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PHENOTYPIC STABILITY IN RATE OF MATURATION OF HETEROZYGOTES FOR INDUCED CHLOROPHYLL MUTATIONS IN TOMATO[1]

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Received May 8, 1956

CONSIDERABLE attention has been directed in recent years to the study of single-gene heterosis. Several workers have presented evidence to indicate that, occasionally, there is a cumulative action of divergent alleles such that the heterozygote is superior to either homozygote. In such cases, then, it appears that heterozygosity *per se* contributes to heterosis.

GARBER and ROWLEY (1927) found that maize plants heterozygous for a recessive, defective endosperm mutation yielded significantly less than did their homozygous normal siblings. WENTZ and STEWART (1927) reported that soybeans heterozygous for a mutation which was semilethal in the homozygous condition, were not significantly different from their homozygous normal siblings with respect to yield. MANGLESDORF (1928) found that a defective endosperm mutation had a slight, but consistently deleterious effect in the heterozygous condition. The traits affected were earliness of blooming, length and weight of ears, and plant height.

KARPER (1930) found that sorghum plants heterozygous for an albino seedling mutation were slightly, but not significantly taller than their homozygous normal sibs. The heterozygotes were also heavier than the homozygotes, but not significantly so. The two genotypes were equal with respect to earliness.

ROBERTSON and AUSTIN (1935) studied three recessive chlorophyll mutations in barley. Single gene heterozygotes were in no way inferior to their respective homozygous normal plants. Two of the genes were linked, and it was found that the double heterozygotes for these loci were significantly superior to the homozygous normal plants with respect to three different measurements of yield.

JONES (1945) investigated five defective mutations in maize. The various heterozygotes were found to yield from 3 to 104 percent better than their respective homozygous siblings and were from 0 to 9 percent taller than homozygotes, depending on the mutation involved.

Of special interest are the investigations of GUSTAFSSON and his co-workers on barley (1946, 1947, 1950a, 1950b, 1951). GUSTAFSSON (1951), working with the induced mutations, viridis and erectoides-29, demonstrated that, although viridis is lethal when homozygous, in the heterozygous condition in the dominant erectoides-

[1] The work described in this paper was performed under the auspices of the Atomic Energy Commission, National Science Foundation, and Purdue Research Foundation. All irradiations were done at Brookhaven National Laboratory. Part of this paper is taken from a thesis presented by the senior author to the faculty of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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29 background, it has a beneficial effect. Plants of the *EE Vv* genotype had a significantly greater number of spikes and kernels, and the kernel weight per plant was also significantly greater in the heterozygotes as compared to the homozygote, *EE VV*. The viridis heterozygote was found to be about 20 percent superior to the normal homozygote in the dominant erectoides-29 background.

STERN *et al.* (1952), investigated the viability of Drosophila heterozygotes for 77 different X chromosome lethals, 37 of which were of spontaneous and 40 of which were of induced origin. A viability index greater than one indicated, in STERN's system, the superiority of the heterozygote. Such indices were obtained for lethals of both origins. Both spontaneous and irradiation-produced lethals gave very similar distributions of viability indices. The latter group showed, however, a slight, but statistically insignificant shift to higher index values. STERN concluded, "No significant difference in viability was found between lethals of control and irradiated origin."

A significant point demonstrated by both GUSTAFSSON and STERN is that heterosis caused by induced mutations is occasionally evidenced. With this knowledge as background, three induced chlorophyll mutations in tomato were studied in order to ascertain what effects, if any, they had on earliness and green weight of heterozygous plants.

MATERIALS

Seeds of two lines of *Lycopersicon pimpinellifolium* were irradiated with either X-rays or thermal neutrons in July, 1954. The lines used (designated 226 and 228) were diploids derived from the same haploid. The seed irradiated was obtained directly from the doubled haploid. In the R_2 generation, two different chlorophyll lethals and one sub-lethal were selected from among the many mutations observed.

(1) Mutant 228 C appeared in the N_2 generation following $2.96 \times 10^{13} N_{th}/cm^2$ irradiation of seed. The homozygous recessive plants were pale yellow-green in color and usually died in the cotyledon stage. If they did survive, they were extremely weak, chlorophyll-deficient plants which never produced flowers. Mutant 228C is seemingly imperfectly transmitted, only 17.6 percent of the N_2 plants being homozygous recessives (70.4 percent of the expected one quarter), and among the 176 normal N_2 plants which were grown in the field, only 84 (i.e. 71.6 percent of the expected number) were heterozygous for the lethal.

(2) Mutant 228D also appeared in N_2 following 2.96×10^{13} N_{th}/cm^2 irradiation of seed. The homozygous recessive was devoid of chlorophyll and was a rich cream in color. Transmission of this mutation appeared normal, since 113 of 179 phenotypically normal N_2 plants were found to be heterozygous (94.2 percent of the expected number). Relatively few mutant seedlings emerged when planted in soil, but when seeds which failed to produce seedlings were uncovered it was found that they had germinated and produced a mutant seedling too weak to push above the surface. In the original N_2 population, 26.4 percent of the seeds either failed to emerge or emerged and were of the mutant phenotype.

(3) Mutant 226 YY occurred in the R_2 generation following 24,000r X-irradiation of seed. This mutation has been designated as "netted" since the chlorophyll of the seedling in the one- and two-leaf stage was concentrated along the vascular tissue,

while the remainder of the leaf was almost devoid of chlorophyll. The homozygous recessive plants survived, set fruit, and bred true for the mutation. In the R_2 generation there were 147 normal green plants and 37 netted plants. Assuming a 3:1 ratio was operative, 46 mutant plants were expected. This deficiency of 9 mutant plants is not statistically significant. However, of 139 normal plants grown to maturity only 76 were found to be heterozygous for netted. Here the deficiency is highly significant. The R_3 progenies of the 76 heterozygotes produced a total of only 247 mutants among 1201 plants, a highly significant deficiency. Only 20.57 percent of the R_3 plants were of mutant phenotype while 20.11 percent of the R_2 plants were netted. These numbers are in agreement, and, along with the deficiency of heterozygotes, indicate defective transmission of the netted mutation.

METHODS

Normal plants of each of the three R_2 progenies were removed to the field in June, 1955. In the case of 226 YY, some homozygous recessives were planted at random among the normals. By means of daily observation, the number of days from seeding to first flower on first inflorescence, first flower on second inflorescence, and first ripe fruit was recorded for each plant. Finally, when most plants had been scored for ripe fruit, all the plants of each R_2 were cut and green weight of each plant was recorded. Fruits were also removed at this time in order to obtain seeds for the progeny tests necessary to distinguish heterozygotes from homozygous normals.

By using the R_2 generation one makes use of the natural randomization of heterozygotes and homozygotes over the area planted. Furthermore, since heterozygotes cannot be distinguished from homozygotes at any time during the period of data-taking, no personal factor can be said to have influenced the results. Finally, it should be noted that the mutants studied were selected at random; no *a priori* knowledge of their behavior was possessed at the time this study was initiated.

ANALYSIS OF DATA

Only one of the mutations studied, chlorophyll lethal 228C, produced any significant effect on the mean of heterozygous plants (table 1 and fig. 1). The homozygous normal plants were consistently earlier than the heterozygotes. The difference in the means was small, being of the order of one to two days. If plant weight was affected by mutant 228C, it was to decrease the mean weight of the heterozygote, the observed difference of 1.50 ounces not being significant.

Mutants 228D and 226YY (tables 2 and 3) produced no statistically significant effect on the means of the heterozygotes either with respect to earliness or plant weight. If 228D had any effect on earliness of the heterozygote, it was a delaying effect (fig. 2). Plants heterozygous for 228D had a mean weight 1.21 ounces greater than that of the homozygotes, but the difference is not significant. Heterozygotes for 226YY were consistently, but not significantly, earlier than their homozygous siblings (fig. 3) and they were considerably heavier (4.69 ounces, table 3). This 4.69 ounce mean weight difference was the largest weight difference obtained but is not quite statistically significant.

It should be mentioned that plants homozygous for mutant 226YY (netted) were

TABLE 1

Analysis of the effect of mutant 228C in the heterozygous condition: mean and variance both affected

Trait measured	Genotype	Number	Mean	Difference in means	Variance	Variance ratio
Days to first flower on first inflorescence	*AA*	92	74.08	1.99*	42.14	1.36
	Aa	84	76.07		30.94	
		176				
Days to first flower on second inflorescence	*AA*	92	80.47	1.02*	14.14	1.52**
	Aa	83	81.49		9.30	
		175				
Days to first ripe fruit	*AA*	92	107.33	1.75*	27.83	1.55**
	Aa	84	109.08		18.00	
		176				
Green weight of plant in ounces	*AA*	89	62.29	1.50	310.69	1.24
	Aa	77	60.79		250.67	
		166				

* Significant at 5% level.

** Significant at 10% level. A two-tailed test is necessitated by the fact that the ratio may meaningfully be less than unity.

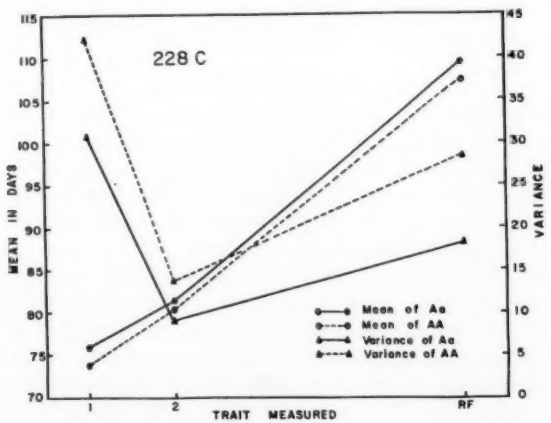

FIGURE 1.—The effect of mutant 228C on the mean and variance of the heterozygote compared to homozygous normal in three earliness traits. In the figure, 1 = first flower, first inflorescence, 2 = first flower, second inflorescence, RF = first ripe fruit, *Aa* = mutant heterozygote, *AA* = homozygous normal.

TABLE 2

Analysis of the effect of mutant 228D in the heterozygous condition: variance only affected

Trait measured	Genotype	Number	Mean	Difference in means	Variance	Variance ratio
Days to first flower on first inflorescence	*AA*	66	75.11	0.87	30.90	1.56*
	Aa	113	75.98		19.77	
		179				
Days to first flower on second inflorescence	*AA*	66	81.23	0.20	16.36	2.71**
	Aa	113	81.43		6.03	
		179				
Days to first ripe fruit	*AA*	58	107.78	1.38	21.20	1.50*
	Aa	110	109.16		14.15	
		168				
Green weight of plant in ounces	*AA*	56	65.02	1.21	396.42	0.91
	Aa	102	66.23		436.45	
		158				

* Significant at 10% level.
** Significant at 2% level.

TABLE 3

Analysis of the effects of mutant 226YY in the heterozygous condition: variance only affected

Trait measured	Genotype	Number	Mean	Difference in means	Variance	Variance ratio
Days to first flower on first inflorescence	*AA*	63	79.51	0.08	6.42	2.10**
	Aa	76	79.43		3.05	
		139				
Days to first flower on second inflorescence	*AA*	63	83.56	0.44	5.99	1.95**
	Aa	76	83.12		3.07	
		139				
Days to first ripe fruit	*AA*	63	111.11	0.18	6.42	2.47**
	Aa	76	110.93		2.60	
		139				
Green weight of plant in ounces	*AA*	57	61.58	4.69	219.86	0.71
	Aa	75	66.27		307.60	
		132				

** Significant at 2% level.

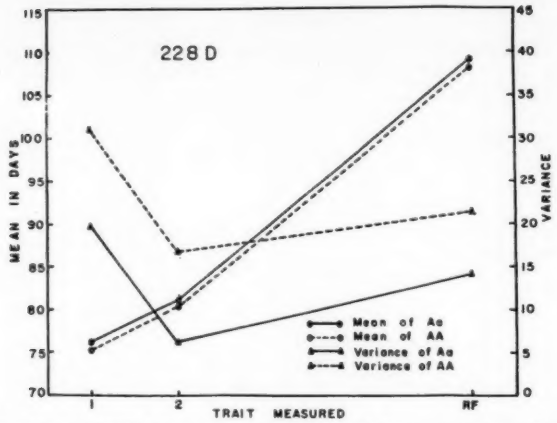

FIGURE 2.—Mutant 228D heterozygotes compared with homozygous normals in three earliness traits. For legend, see figure 1.

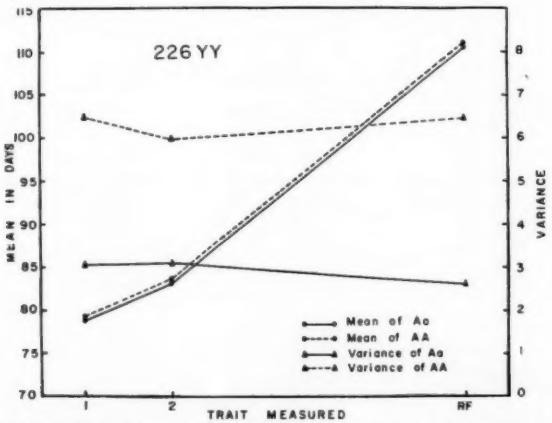

FIGURE 3.—Mutant 226YY heterozygotes and homozygotes show widely different variances but approximately the same mean. For legend, see figure 1.

significantly later than either their homozygous normal or heterozygous sibs. The difference here was of the order of four to five days. The mean number of days to first flower on first inflorescence was 83.68, while the mean second inflorescence measurement was 88.54 days for the mutants. No further data were taken on the mutant plants since they did not have ripe fruit at the time the *AA* and *Aa* plants were cut and weighed.

Phenotypic stability

The most striking feature of the data presented in tables 1, 2, and 3 is that whenever a significant difference exists between the variance of the heterozygote and that

of the homozygote, the former has the *smaller* variance. It is especially noteworthy that all three mutants behave in the same manner.

In view of the fact that heterozygote means are not markedly different from those of homozygotes, it is interesting to find the heterozygote variances for the three earliness traits consistently lower and usually significantly lower. This greater phenotypic stability of "single gene" heterozygotes is suggestive of the greater uniformity of hybrids and seems to indicate a genotype-environment interaction of major proportion. One can reasonably assume that the edaphic conditions to which these plants were exposed varied considerably and that homozygotes gave phenotypic response to soil differences while heterozygotes more consistently produced the same phenotype in spite of these environment differences.

DISCUSSION

The striking phenomenon of phenotypic stability in rate of maturation of heterozygotes for chlorophyll mutations is in contrast to the results of GUSTAFSSON (1946) who emphasized the "plasticity" of the heterozygote: "Eighty *albina* 7 heterozygotes gave an average ear number of 4.92 with $\sigma = 2.54$ and V_1 (the variation coefficient) $= 51.62$. Similarly 39 homozygotes produced 4.67 ears per plant, σ equalling 1.77 and V_2 37.90. The proportion of V_1 to V_2 is 1.36." GUSTAFSSON then listed additional examples in his own data and analyzed in a like manner the data of ROBERTSON (1932) and ROBERTSON and AUSTIN (1935). Almost always, in these data, the variability of the heterozygotes exceeded that of the homozygotes.

Finally, GUSTAFSSON (1946) stated: "We may therefore conclude that the variability is substantially greater in plants having one lethal in heterozygous state than in plants of a pure line." GUSTAFSSON's barley mutations, when present in heterozygous condition, made length of ears, number of culms, and weight of seeds more variable, while the same mutations inhibit chlorophyll formation in homozygous state. These conclusions are diametrically opposed to the ones which must be drawn from data presented in this paper.

LERNER (1954) emphasizes that GUSTAFSSON failed to analyze the data of MANGLESDORF (1928) and KARPER (1930) in the manner in which he analyzed the barley data. Had he done so, GUSTAFSSON would have found that the maize and sorghum data afford no support to his hypothesis.

It is conceivable, however, that the present examples of "single gene" heterozygotes do not, in fact, involve single loci. That is, the visible chlorophyll mutation may be associated with a simultaneously induced, linked mutation, affecting earliness of maturity.

MULLER has long been concerned with the possibility of the simultaneous production of linked mutations by ionizing radiations. PATTERSON and MULLER (1930) irradiated Drosophila larvae of the *delta-49 f* stock and mated the resulting males to *y* attached-X females. The male progeny from this mating had the irradiated *delta-49 f* X chromosome and a Y chromosome from the attached-X female. Any visible mutations induced in the *delta-49 f* X chromosome would thus be exposed. Of 2651 males examined only two had mutations, but the one fly had *two* mutations: a reverse mutation at the forked locus and linked to it, a new mutation, spectacled, at the

lozenge locus. MULLER (1932) stated that the probability that these two mutations were of independent origin is only one in 883.

MULLER (1932) also observed an induced mutation at the scarlet locus, which was associated with an induced lethal linked to it with 0.1 percent recombination. MULLER felt that these two mutations must have been simultaneously induced, since the probability was less than one in 1000 for the two closely linked mutations to be produced independently.

MULLER (1932) was especially concerned with the simultaneous induction of mutations at the scute locus and linked lethal effects. He observed that perhaps more than 25 percent of the induced scutes were connected with a lethal. He also observed an induced scute linked to a mutation affecting the ommatidia. MULLER finally concluded that ". . . there can then be no reasonable doubt of the tendency of induced gene mutations . . . to occur in localized groups."

NISHINA and MORIWAKI (1941) designed an experiment to detect the production of linked mutations by fast neutrons. They irradiated males and mated them to *ClB* females. Those F_2 progenies yielding no males were known to contain at least one lethal. By mating the non-Bar F_2 females, which were heterozygous for the irradiated X chromosome, to wild type males, and then observing the F_3 sex ratio, they could detect whether more than one lethal had been induced. The F_3 sex ratio should have been two females to one male if only one lethal was present. However if two lethals were induced, and if there was an appreciable amount of recombination between them, then the ratio would approach four females to one male as the linkage approached free recombination.

Of 18 lethals so tested, six were found to give F_3 ratios indicative of the presence of two linked lethals, while two other lethals were found to be linked to visible mutations. Thus, in about forty percent of the cases two linked mutations appeared to be simultaneously induced.

In view of these facts it does not seem unreasonable to assume that the present study involves simultaneously induced, linked mutations rather than mutations at single loci. The question immediately arises as to how such linkage would affect the interpretation of the data, especially with reference to the relative stability of heterozygotes in their rate of maturation.

Linkage model

It is possible to define a model based on linkage of two induced mutations which can account for the smaller variance of heterozygotes for the chlorophyll mutations. This model, with minor alterations, will fit all three of the mutations investigated. Assume that mutation occurs at locus *A* affecting chlorophyll production and at linked locus *B* affecting earliness. The *A-a* locus has no effect on the mean or variance of the earliness traits measured, but homozygous *aa* plants are lethal. All of the effect on mean and variance is contributed by the *B-b* locus.

In this model p equals the recombination frequency between *A* and *B* in coupling phase. A value of p greater than 0.50 indicates repulsion linkage. The term q equals $(1 - p)$. The additive genetic effect of *B* is d and that of *b* is $-d$, while h equals the contribution of dominance.

The mean (μ) and variance (σ^2) of AA plants may be expressed as follows:

$$\mu_{AA} = (1 - 2p)d + 2pqh. \quad (1)$$

$$\sigma^2_{AA} = 2pq[d^2 - 2(1 - 2p)dh + h^2(1 - 2pq)]. \quad (2)$$

The same parameters when calculated for plants heterozygous for the chlorophyll mutations are:

$$\mu_{Aa} = (1 - 2pq)h. \quad (3)$$

$$\sigma^2_{Aa} = 2pq[d^2 + h^2(1 - 2pq)]. \quad (4)$$

The variance ratio, $F = \frac{\sigma^2_{AA}}{\sigma^2_{Aa}}$, may be obtained from (2) and (4).

$$F = 1 - \frac{2(1 - 2p)dh}{d^2 + h^2(1 - 2p + 2p^2)}. \quad (5)$$

Now, if dominance is complete ($d = h$),

$$F = 1 - \frac{(1 - 2p)}{1 - p + p^2}. \quad (6)$$

Expressing h as a constant fraction of d, the value of F is determined solely by p. When there is no dominance ($h = 0$), $F = 1.0$, regardless of the value of p. It can also be seen from (5) and from figure 4 that if $p = 0.50$ (independent assortment), $F = 1.0$, regardless of the values of d and h. Therefore, it should be emphasized that some degree of dominance ($h > 0$) and repulsion linkage ($p > 0.50$) must be assumed to obtain $F > 1.0$. The data thus permit acceptance of only this very specific case of the linkage model. The maximum value of F is 2 when $d = h$ and $p = 1.0$; i.e. $F = 2$ when linkage is complete in repulsion phase and there is complete dominance. These relationships are shown in figure 4. In this figure the effects of partial dom-

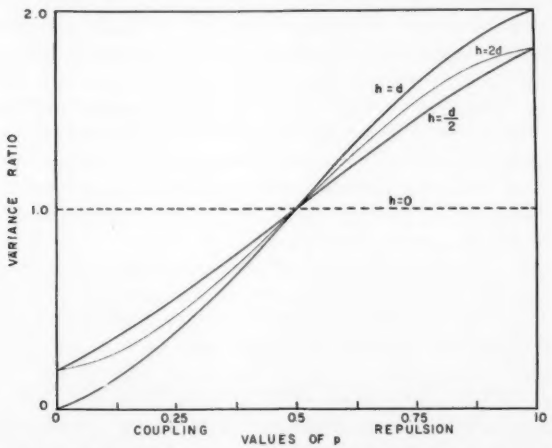

FIGURE 4.—The effect of recombination (p) and degree of dominance (h) on the variance ratio, $F = 1 - \frac{2(1 - 2p)dh}{d^2 + h^2(1 - 2p + 2p^2)}$. When $h = d$, dominance is complete; when $h = 0$, there is no dominance. For overdominance, $h = 2d$; and for partial dominance, $h = \frac{d}{2}$.

TABLE 4

A demonstration of how linkage with 1% recombination could make heterozygotes for chlorophyll mutations have smaller variance than normal homozygotes

Genotype	Frequency* (f)	Phenotypic value (x)	fx	fx^2
AA				
BB	0.0001	0	0	0
Bb	0.0198	0	0	0
bb	0.9801	2	1.9602	3.9204
	1.0000		1.9602	3.9204
	Mean[1] = C + 1.9602; Variance = 0.0780			
Aa				
BB	0.0099	0	0	0
Bb	0.9802	0	0	0
bb	0.0099	2	0.0198	0.0396
	1.0000		0.0198	0.0396
	Mean[1] = C + 0.0198; Variance = 0.0392			

* Frequency adjusted to total 1.0000.

[1] C = contribution of rest of genotype to mean phenotypic value.

inance ($h = d/2$) and overdominance ($h = 2d$) are also shown. Figure 4 demonstrates that as h approaches zero or as h gets very large, F approaches one. Any value of $F > 2$ experimentally obtained must be attributed to sampling error under the assumptions of this model.

A specific example of how this model compares with experimental data is summarized in table 4. Assuming that the seed irradiated was of genotype *Ab/Ab*, mutations could occur at both loci in one chromosome such that the R_1 plant would have genotype *Ab/aB*. If there is one percent recombination between the two loci in this repulsion linkage and if all *aa* plants die, then the remaining genotypes will occur in the frequencies given in table 4. In this example, the variance of *AA* plants is 1.99 times that of *Aa* plants. Since dominance for earliness is assumed (as was found by BURDICK 1954), the *AA* plants are later than the *Aa* plants. This is similar to the case of mutant 226YY.

The model fits the cases of 228C and 228D if one assumes dominance for lateness. By assigning a phenotypic value of 2 to *BB* and to *Bb* and a value of 0 to *bb*, the mean of *AA* (C + 0.0398) is less than that of *Aa* (C + 1.9802). The variances of the two groups (*AA* and *Aa*) remain the same as in table 4.

Action of irradiation in producing clusters of mutations

The linkage hypothesis thus becomes a very interesting way of explaining the data in this experiment, and especially the phenotypic stability of heterozygotes relative to homozygotes. Obviously the validity of the model rests on the assumption that ionizing radiations can produce linked mutations of the nature discussed above.

Recent experiments of MULLER (1954b) have led to the conclusion that neutrons

are more effective than are X-rays in producing linked mutations. The neutron/X-ray effectiveness for producing separately detectable sex-linked recessive lethals was 2.4 while the ratio for visible mutations at specific loci was about 4. This was interpreted to mean that neutrons produce lethals which are so closely linked that they are not detected as separate events. This difficulty is, of course, not encountered in detecting visible mutations at specific loci.

MULLER (1954a), pointed out that the lesser efficiency of neutrons as compared to X-rays in inducing X chromosome lethals is well established. Neutrons produce such lethals with only about two-thirds the efficiency of X-rays. MULLER suggested that probably the same number of mutations are produced, but that in the case of sex-chromosome lethals, one third of the lethals go undetected because of their close linkage to other lethals.

The question arises as to what mode of action irradiation takes in producing, simultaneously, mutations at linked loci. MULLER (1932) stated that clusters of mutations must be produced by some indirect effect of irradiation. MULLER (1940) reaffirmed the view that linked mutations or breaks must be produced by the spreading effect of a single ionization to both the neighboring points in question.

If the production of mutations by ionizing radiations is indirect and due to the action of active radicals produced in the path of an ionizing particle (LEA 1947), then the induction of more than one mutation per track is conceivable. LEA stated that H radicals are produced 15 mμ from the path of the particle. If there are about 400 genes and 100 crossover units per chromosome in the tomato and if the average somatic chromosome length is 2μ, then one crossover unit is equivalent to 4 genes or 20 mμ of chromosome length. Thus, with a spread of 30 mμ, perhaps as many as 6 genes could be affected by one ionization. This mechanism could account for mutations at closely linked loci.

NISHINA and MORIWAKI (1941) interpreted their results on purely physical grounds. The heavy ion density of the recoil protons produced by fast neutron bombardment was considered to be responsible for grouping of mutations within chromosomes.

A similar interpretation was given by MULLER (1956): "The most probable interpretation of these cases [of induced linked mutations] is that two ionizations of the same delta-ray cluster or primary electron track tail both chanced to be mutagenic. The same sort of thing seems to occur in much greater relative abundance with neutron treatment, as is to be expected on this interpretation."

On the basis of these theoretical considerations and the available experimental evidence it does not seem unreasonable to offer the linkage model as a possible interpretation of the data of this experiment. The greater phenotypic stability of heterozygotes for chlorophyll mutations may be interpreted on the basis of the stabilizing action of a single locus or by the action of two linked mutations, one a chlorophyll mutation, the other a mutation affecting earliness. A test of the linkage hypothesis can be devised, since, if this hypothesis is correct, some of the R_2 heterozygotes (*Aa BB* or *Aa bb*) should produce heterozygous progeny with the same variance as that of their homozygous progenies. Other R_2 heterozygotes (*Aa Bb*) should repeat the performance observed in R_2. If the linkage hypothesis, so tested,

proves untenable, then an explanation based on the stabilizing action of a single locus would seem in order.

SUMMARY

1. Three radiation-induced chlorophyll mutations in *Lycopersicon pimpinellifolium* were studied in the heterozygous state to determine their effects on earliness and green weight of plants. The earliness traits were number of days to first flower, first inflorescence; days to first flower, second inflorescence; and days to first ripe fruit. None of the mutations produced dominant morphological effects. Heterozygotes for the chlorophyll mutations were compared to their homozygous normal siblings.

2. Only mutant 228C produced statistically significant effects on the mean of heterozygotes. In this case heterozygous plants were later than their homozygous normal sibs for all three measures of earliness.

3. All three mutations, however, produced effects on the variance, such that the variance for the three earliness measures was significantly *smaller* for the heterozygotes than for homozygous normal sibs.

4. The greater phenotypic stability of heterozygotes may be due to the action of a single locus. This interpretation indicates a genotype-environment interaction of major proportion.

5. Linkage of two induced mutations, one affecting chlorophyll production and lethal in homozygous condition, the other affecting earliness and contributing the observed effects on mean and variance might also account for phenotypic stability of heterozygotes.

6. Since independent production of linked mutations is a rare event and since linked mutations could be very close together (one crossover unit or less), clusters of mutations may have been produced by irradiation.

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AN INFLUENCE OF PLOIDY ON THE TIME OF EXPRESSION OF DOMINANT LETHAL MUTATIONS IN HABROBRACON[1]

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WHEN Habrobracon eggs are X-rayed at metaphase I and subsequently fertilized, the decreased hatchability is attributed to dominant lethal mutation, in the same sense in which the term is used when sperm are irradiated. This is justified by WHITING's demonstration (1946, 1948, 1949, 1950, 1955) that cytoplasmic injury is not lethal at the doses employed. In unfertilized eggs, which develop as haploids, both dominant and recessive lethals are expressed, whereas only the dominant effects are expressed in the diploid zygotes. Hence the viable proportion should, in general, be increased if the eggs are fertilized after irradiation. An early experiment of WHITING (1945b) showed dominant lethality to be the most frequent consequence of X irradiation of the oocytes, and the expected difference due to recessive lethals was not statistically significant. In further experiments, to be reported here, significant differences have been found, and these can theoretically be used to estimate the frequency of recessive lethals. This frequency can also be obtained by HEIDENTHAL's method (1952), a test for heterozygosity of the F_1 females. Thus the recessive embryo lethals induced in oocytes can be estimated from two different data; first, from the difference in hatchability of fertilized and unfertilized eggs, and second, from the proportion of F_1 females found by later testing to be heterozygous for such recessive lethals. When these two estimates are made within the same experiment, a curious discrepancy is noted: the apparent frequency of recessive embryo lethals is much greater by the first than by the second method.

It will be shown that the disagreement results from the presence of a class of *dominant* lethals expressed before hatching in haploids, but after hatching in diploids. This leads to underestimation of the dominant lethality in diploids, and a corresponding overestimate of recessive lethals when the hatchabilities of haploids and diploids are compared after irradiation in metaphase I. When eggs are irradiated while in prophase, no such discrepancy is observed. This indicates a somewhat different basis for dominant lethality, depending on the stage irradiated. Aside from the delayed expression of certain dominant lethals, no anomalous effect of the unirradiated chromosome set need be assumed in order to explain the results. Thus it is shown that the introduction of an unirradiated chromosome set, within the time limitations of the technique, does not change the frequency of dominant or recessive lethal mutations in an irradiated set. Although this point may at first seem trivial, on closer scrutiny it

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is by no means self-evident, and would ordinarily be rather difficult to test by experiment.

MATERIALS AND METHODS

Experimental procedures for studying lethal mutations in Habrobracon have been given in a number of previous publications (e.g., WHITING 1945b; HEIDENTHAL 1952, 1953). The experiments are based on the fact that unmated females produce haploid sons only, whereas in mated females about two thirds of the eggs are fertilized and produce diploid daughters. This proportion is sufficiently constant to warrant its assumption, for practical purposes, in experiments on egg hatchability where determination of the sex ratio is not feasible. Eggs in the uterine sac are arrested in late metaphase I until they are laid, then anaphase I proceeds within a few minutes. All eggs laid within six hours after irradiation are known to have been in metaphase I at the time of treatment.

Well-fed adult virgin females of wild type stock No. 33 of Habrobracon were stored in empty shell vials at 30°C for 12 to 14 hours prior to irradiation. Immediately after irradiation they were transferred to host caterpillars (Ephestia). Eggs laid during the first six hours on the host were used in the experiments concerning irradiation in metaphase I. When fertilization occurs, it coincides with oviposition; hence the elapsed time between irradiation and fertilization varies from about 30 minutes to six hours. Experiments on irradiation of prophase I were done with eggs laid between the eighth and twelfth hours on hosts. Matings to stock No. 17 o^i males were made, where indicated, immediately after irradiation and before transfer to host.

In most experiments X-rays were administered with a G. E. Maxitron unit operated at 250 kvp, 30 ma, with 3 mm of aluminum filtration. The eggs in metaphase I were exposed with a target distance of 39 cm and dosage rate of 330 to 340r/minute. For eggs exposed in prophase I the target distance was 16.5 cm and dosage rate 1888r/minute. A previously described X-ray facility at the Marine Biological Laboratory, Woods Hole, was used in some experiments (WHITING 1945b). The dose rates in the latter were 600r/minute and 2650r/minute and distances 30 cm and 14.4 cm for metaphase I and prophase I, respectively.

METHODS OF ESTIMATING LETHALS

The first, or F_1, method for estimating recessive embryo lethals is by comparison of the hatchability of eggs from unmated and mated females. The viable proportion of unfertilized eggs, V_u, is directly observed when the females are unmated. That of fertilized eggs, V_f, is given by

$$V_f = \frac{m - V_u(1 - f)}{f}, \tag{1}$$

where m is the hatchability of eggs from mated females and f is the proportion fertilized, assumed to be 0.66 in experiments on hatchability, where direct determination of the sex ratio is impracticable.

Since surviving larvae from unfertilized eggs carry neither dominant nor recessive embryo lethal mutations,

$$V_u = (1 - D)(1 - r), \tag{2}$$

where D is the frequency of chromosome sets having at least one dominant and r the frequency of those having at least one recessive lethal. Fertilized eggs that hatch are simply those without a dominant embryo lethal;

$$V_f = 1 - D. \tag{3}$$

Thus the frequency of genomes bearing at least one recessive embryo lethal should be given by

$$r = 1 - (V_u/V_f), \tag{4}$$

or, from equations (1) and (4),

$$r = \frac{m - V_u}{m - V_u(1 - f)}. \tag{5}$$

When adult survival data are available, the same method can be used to estimate the frequency, r', of genomes bearing at least one recessive lethal expressed at *any* stage of development. For this purpose the foregoing equations are applicable where V'_u, m', and V'_f for imagos correspond to V_u, m, and V_f for embryos. In such experiments the values of f and V'_f can also be computed from the sex ratio. Let q represent the ratio of surviving adult females to total eggs. Then,

$$V'_f = q/f. \tag{6}$$

By substitution in equation (1), etc.,

$$f = (q - m' + V'_u)/V'_u, \tag{7}$$

and

$$r' = 1 - (q - m' + V'_u)/q. \tag{8}$$

The second, or F_2, method is to isolate unmated F_1 females and determine with what frequency these are heterozygous for recessive lethals (HEIDENTHAL 1952). The haploid eggs of a wasp that is heterozygous for one or more recessive embryo lethals will have a hatchability of 50% or less. The procedure for r' by the F_2 method is the same as for r except that the criterion of adult survival is substituted for that of hatchability. The detection of recessive lethals in the F_2 is clear cut; sufficient eggs are obtained from each female to establish the gametic ratio, and scoring is facilitated by the fact that F_2 male progeny carrying the same lethal usually die at the same stage. The F_2 method is, therefore, potentially more accurate than the F_1 where individual eggs represent presence or absence of different lethals and where occasional death due to environmental cause interferes more seriously with ratios.

Both F_1 and F_2 procedures were followed within the same experiment; that is, the females used in obtaining the F_2 data were the survivors represented by V'_f in the corresponding F_1.

RESULTS

The frequencies of recessive lethal mutations induced in eggs during prophase I, when they are relatively resistant to radiation, are shown in table 1. The frequencies, r, of recessive embryo lethals by the F_1 and F_2 methods are in excellent agreement.

TABLE 1

Incidence, r, of eggs carrying recessive embryo lethal mutations induced at prophase I, estimated by the F_1 and F_2 methods. A. Irradiated in air B. Irradiated in nitrogen

Dose (r)	F_1 Method			F_2 Method		P_i
	V_u viable/total; from unmated ♀♀	m viable/total; from mated ♀♀	r_1 from equation (5)	Heterozygous ♀♀/no. of ♀♀ tested	r_2 proportion of females carrying recessive lethals	Appr. significance level of $\lvert r_1 - r_2 \rvert$
A. 5,000	87/102	210/238	0.050	2/157	0.013	0.59
15,000	76/128	240/374	0.109	14/134	0.105	0.97
15,000	211/537	232/531	0.145	17/118	0.144	0.99
25,000	34/185	33/162	0.141	3/20	0.150	0.98
B. 15,000	212/248	229/278	−0.058	2/127	0.016	0.21
25,000	63/132	69/136	0.087	6/47	0.128	0.82

TABLE 2

Incidence, r, of eggs carrying recessive embryo lethal mutations induced at metaphase I, estimated by the F_1 and F_2 methods. A. Eggs laid within 6 hours after treatment. B. Eggs held for 24 hours after treatment before oviposition. C. Irradiated in nitrogen

Dose (r)	F_1 Method			F_2 Method		P_i
	V_u viable/total; from unmated ♀♀	m viable/total; from mated ♀♀	r_1 from equation (5)	Heterozygous ♀♀/no. of ♀♀ tested	r_2 proportion of females carrying recessive lethals	Appr. significance level of $\lvert r_1 - r_2 \rvert$
A. 500	426/758	947/1492	0.164	18/312	0.058	0.023
871	151/559	205/577	0.323	16/99	0.162	0.071
875	236/933	357/1100	0.300	25/127	0.197	0.18
1100	89/704	180/866	0.494	21/138	0.152	<0.001
1100	159/1061	158/748	0.383	19*/113	0.168	0.025
B. 1100	12/113	36/172	0.595	—	—	—
C. 1100	352/644	923/1511	0.151	32/620	0.052	0.052

* From separate experiment.

Irradiation in nitrogen reduced the effectiveness of the X-rays, but did not alter the agreement between F_1 and F_2 results. In one prophase experiment (see table 3) the frequencies, r', of the total recessive lethals, based on adult survival, are compared. Here, also, the F_1 and F_2 methods give similar results.

A contrasting result is seen in table 2, where the embryo recessive lethal frequencies are given for eggs irradiated at metaphase I. The value of r estimated by the F_1 method is always higher than that by the F_2 method. In one experiment the females were held without hosts for 24 hours after irradiation. During this period the eggs are retained and those irradiated in metaphase I are laid in the first six hours after hosts are finally supplied. With these retained eggs (table 2, B) the F_1 method gave an excessive value for r, just as in the other experiments, which shows that the difference between the results of irradiation in prophase and metaphase is not caused by the delay in obtaining the eggs X-rayed in prophase. In another experiment metaphase I eggs were irradiated in an atmosphere of nitrogen. The dose of 1100r in nitrogen, as shown in table 2 (C), is equivalent to 500r in air. The discrepancy be-

TABLE 3

Recessive lethal mutations estimated from adult survival

Stage irradiated and dose (r)	F_1 Method						F_2 Method	
	V'_u	m'	q (♀ survivors/ total eggs)	f from eq. (7)	r' from $f = 0.66$, eq. (5)	r' from eq. (8)	r'	No. of ♀♀ tested
Metaphase I 871	123/558	155/577	0.175	0.586	0.252	0.280	0.242	99
875	172/931	242/1099	0.129	0.508	0.224	0.272	0.252	127
1,100	126/1174	98/748	0.094	0.650	0.262	0.255	0.239	113*
Prophase I, 15,000	150/537	173/531	0.226	0.642	0.214	0.208	0.237	118

* Data obtained from a separate experiment.

tween the F_1 and F_2 estimates of r is also the same as would have occurred with a dose of 500r in air. Although the reality of the difference between F_1 and F_2 results seems obvious on inspection of table 2, significance levels were approximated in order to test this impression. Because of the manner in which r is computed by the F_1 method, the significance levels cannot be exact. The variances of the F_1 estimates of r were approximated by the asymptotic variance formula for the ratio in equation (5), whereas those for the F_2 were computed exactly. Significance levels were then obtained by STUDENT's t formula. The approximate P values suggest that the F_1 and F_2 results are in the main significantly different in table 2, and not in table 1. The overall significance levels by FISHER's method (1946) are >0.963 for table 1 and <0.001 for table 2.

In three experiments on metaphase I eggs and one experiment on prophase I eggs the total recessive lethals, r', expressed at any time in development, were scored by the use of adult survival data in the manner already indicated. The results of these experiments are shown in table 3. The values of r' determined by F_1 methods do not differ substantially from those by the F_2, even after metaphase irradiation, whereas the values of r in the same experiments show the large discrepancy already indicated in table 2. The same conclusion is reached whether r' is computed according to equation (5) with the assumed value of $f = 0.66$, or from equation (8) with the observed sex ratio. Evidently the difference in mortality between haploids and diploids is greater at the time of hatching than at the time of eclosion. In other words, the difference in hatchability between fertilized and unfertilized metaphase-irradiated eggs must be partly offset by an increased mortality of the diploids during postembryonic stages of development. This inference is supported by direct observation of the mortality at different stages in the same experiments. The results are given in table 4, where it can be seen that the larval mortality is higher in the diploid than in the haploid. It should be noted that deaths known to be accidental, e.g., by dislodgement from the host, have been discounted from the totals in all tables.

DISCUSSION

Although recessive lethal mutations are emphasized in the presentation of methods and results, the striking and fundamental point is the difference between dominant *embryo* lethal mutation rates in haploids and diploids. When recessive embryo lethal

TABLE 4

Mortality at different postembryonic developmental stages. Same experiments as in table 3

Dose (r)	Larval lethals/larvae				Pupal lethals/pupae			
	Unmated		Mated		Unmated		Mated	
	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion
Metaphase I								
871	18/150	0.120	31/202	0.153	9/132	0.068	16/171	0.094
875	34/234	0.145	83/355	0.234	28/200	0.140	30/272	0.110
1,100	28/171	0.164	41/158	0.259	17/143	0.119	19/117	0.162
Prophase I								
15,000	48/211	0.227	38/232	0.164	13/163	0.080	21/194	0.108
Controls								
0	46/672	0.069	50/545	0.092	27/626	0.043	18/495	0.036
0	6/116	0.052	3/102	0.029	1/110	0.009	3/99	0.030

rates were estimated by equation (5), it was at first taken for granted that the dominant rates in haploids and heterozygotes were the same. As soon as F_2 data for recessive embryo lethals were obtained for eggs irradiated in metaphase I it became evident that embryo lethality in the haploid eggs could not be fully accounted for by combining the dominant rates from diploid hatchability and the recessive rates obtained from diploids by the F_2 method. In an attempt to explain this discrepancy, three hypotheses were considered: first, that semilethals or complementary lethals contribute substantially to the F_1 result, but are frequently overlooked by the F_2 method; second, that the undamaged chromosome set, brought in through fertilization, might prevent the consummation of latent damage to the irradiated set; third, that fertilization does not change the frequency of dominant or recessive lethals, but postpones the expression of some of the dominant lethals until postembryonic stages. Only the third hypothesis is consistent with the agreement of F_1 and F_2 methods when the criterion of adult survival is used. Moreover, only this hypothesis demands a higher mortality of diploids in postembryonic stages. Therefore, it seems reasonable to attribute the discrepancy between F_1 and F_2 results largely, if not entirely, to delayed expression of dominant lethals in the diploids.

When the delayed dominant mutations are taken into account, equation (2) becomes

$$V_u = (1 - D)(1 - D_e)(1 - r), \tag{9}$$

where D_e is the frequency of delayed dominant lethal mutations and r is the value in agreement with that obtained by the F_2 method. It must be emphasized that D_e is not necessarily the frequency of all dominant lethals subject to delay, but only of those that can be delayed until after hatching. From equations (3) and (9),

$$D_e = 1 - \frac{V_u}{V_f(1 - r)}. \tag{10}$$

Values of D_e, computed from equation (10) by means of r values obtained by the F_2 method, are given in table 5. For metaphase irradiation D_e is large, from $\frac{1}{3}$ to $\frac{2}{3}$ the value of D. For eggs treated in prophase, the values of D_e are best interpreted as

TABLE 5

Frequencies of dominant (D) *and conditionally delayed dominant lethal mutations* (D_e) *induced in eggs at metaphase I and prophase I. Calculated from data in tables 1 and 2*

Stage irradiated and atmosphere	Dose (r)	D	D_e
Prophase I, Air	5,000	0.094	0.038
	15,000	0.328	0.005
	15,000	0.541	0.001
	25,000	0.784	−0.010
Nitrogen	15,000	0.192	−0.075
	25,000	0.472	−0.046
Metaphase I, Air	500	0.352	0.113
	871	0.597	0.193
	875	0.636	0.129
	1,100	0.749	0.403
	1,100	0.755	0.273
Held 24 hr	1,100	0.737	0.514
Nitrogen	1,100	0.358	0.105

random deviations from zero. In view of the dependence of the delayed dominant effect on the meiotic stage irradiated, the effect should be correlated with radiation-induced changes peculiar to the metaphase. A provisional explanation for this stage dependence will be suggested after a consideration of possible cytoplasmic effects and of the observable cytological abnormalities.

The lethal effects of X-rays in the experiments on metaphase eggs are most economically explained in terms of nuclear damage; the effects on the cytoplasm, at doses less than 15,000r, are believed to be without influence (WHITING 1946, 1948, 1949, 1950, 1955). Strong evidence for this view is provided by the occurrence of androgenetic males, which result from failure of the normal migration and fusion of the female pronucleus in irradiated eggs. For instance, the surviving fraction of eggs X-rayed in metaphase is less than 0.003 at a dose of 2500r, but metaphase eggs that have received many times this dose can undergo completely normal development as haploid males bearing the paternal genome. This demonstrates the competence of the heavily irradiated cytoplasm to support normal embryogenesis. Androgenesis is not an interfering factor in the estimation of induced lethal mutation because, within the dose range suitable for mutation studies, the female pronucleus completes its migration and fusion with the male pronucleus. It may be added that the present experiments indicate no appreciable role of irradiated cytoplasm in producing dominant lethal mutations. If the cytoplasm were mutagenic, more mutations would be expected from its action on two sets of chromosomes than on one, but no such effect is observed when fertilization follows irradiation. This is consistent with WHITING's observation (1950) that induced visible mutations do not appear in androgenetic males that develop with irradiated cytoplasm. Finally, on a nuclear basis alone, one would expect that eggs irradiated shortly *after* fertilization would be much more sensitive than unfertilized eggs at the corresponding stage since the chances of inducing dominant lethal mutation should be approximately doubled in the diploid. This expectation is borne out in the experiments of CLARK and MITCHELL (1952) on

irradiation of early embryos. At very high doses, where development never occurs except through androgenesis, the viability is probably decreased by cytoplasmic damage, as evidenced by a decline in the frequency of androgenetic males at doses above 15,000r at metaphase I (WHITING 1950). It is not known whether cytoplasmic damage contributes to lethality in prophase-irradiated eggs, in which androgenetic males apparently do not occur, even with high doses. As will be seen, subvisible effects of some sort are required to explain part of the lethality of prophase irradiation.

The gross chromosomal effects that can be observed after irradiation of Habrobracon oocytes differ in type and relative frequency depending upon the stage irradiated (WHITING 1945a, b). Irradiation in metaphase I produces chromosome fragments, but no bridges, in anaphase I. Bridges appear in anaphase II and, probably as a result of sister union, in the mitotic divisions prior to death of the embryo. This suggests that the terminal deletions may be followed by breakage-fusion-bridge cycles (MCCLINTOCK 1938). These effects are assumed to be lethal since grossly deficient genomes are not found among the viable progeny. The quantitative correlation between hatchability and absence of fragments or bridges is fairly good although the hatchability is somewhat lower than would be expected to result from the chromosomal aberrations alone (WHITING 1945b). After irradiation in prophase, however, the most striking feature is the high frequency of apparently normal division. Some bridges are seen in anaphase I and II, and in cleavage, but even at 42,000r where lethality is about 99%, the frequency of apparently normal meiosis is over 85% (WHITING unpublished). Thus the observable chromosomal anomalies can account for a large proportion of the dominant lethality from irradiation in metaphase, but for only a small proportion in prophase. It may be surmised that the type of dominant lethal capable of delayed expression in the diploid is associated with chromosome breakage. This idea is strengthened by a further observation that largely accounts for the remaining lethality by a process not subject to delay.

A distinctive type of dominant lethal with a constant time of expression has been described by VON BORSTEL (1955) in Habrobracon and is believed to represent a defect in deoxyribonucleic acid synthesis. Mitosis ceases after six to seven divisions, and at this time the nuclei are anomalously Feulgen negative. They subsequently enlarge up to 200 times their normal volume. This type of lethality (FNS) appears after irradiation of oocytes in either prophase or metaphase and also after irradiation of sperm. Table 6 shows its incidence in eggs from mated and unmated females ir-

TABLE 6

Frequency of irradiated eggs dying at the sixth or seventh cleavage and having Feulgen-negative swollen nuclei (FNS)

Stage irradiated and dose (r)	Females	FNS/Dead eggs		FNS/Total eggs	
		Number	Proportion	Number	Proportion
Prophase I, 15,000	Mated	227/299	0.759	227/531	0.428
	Unmated	236/326	0.724	236/537	0.441
Metaphase I, 1,100	Mated	310/454	0.683	310/576	0.538
	Unmated	425/639	0.665	425/750	0.567

radiated in metaphase or prophase. It is clearly nuclear, since it occurs after metaphase irradiation with doses that do not damage the cytoplasm. Since FNS accounts for an even larger share of the lethality after prophase irradiation, at a dose that produces few detectable chromosomal aberrations, it appears not to be a secondary consequence of gross aneuploidy. This notion is supported by the observation that eggs from triploid females, inviable because of aneuploidy, usually die at stages later than the seventh cleavage and do not show the swollen, Feulgen-negative nuclei (VON BORSTEL and PARDUE 1956). Thus the phenomenon is difficult to reconcile with the usual concepts of dominant lethality. Although the underlying basis of FNS is not known, it is clear that its frequency and time of expression are the same in fertilized as in unfertilized eggs.

Even if this distinctive syndrome, FNS, is assumed to be completely independent of observable chromosome breakage, it is doubtful that both effects combined can fully account for the lethality after irradiation in prophase. The prophase data in table 6, for 15,000r, together with WHITING's observations on chromosome breakage at 42,000r, will not explain the total lethal effect at the lower dose; hence, in the absence of more extensive observations, it seems reasonable to assume a class of embryos that die without either observable abnormality.

In conclusion, at least three variants of the cytological picture in dominant lethality may be recognized: first, swollen, Feulgen-negative nuclei; second, chromosome bridges and fragments; third, no observable abnormality. After metaphase irradiation the first and second are predominant, but after prophase, the first and third. Since the Feulgen-negative nuclear syndrome is unaffected by fertilization, the lethals subject to delay are believed to be included among those caused by chromosome breakage. The introduction of a normal chromosome set covers all deletions in the maternal set, thus converting an absolute deficiency into a condition of genic imbalance. It is a plausible assumption that initial genic imbalance would often permit the embryo to reach a later stage of development than would be reached in complete absence of some portion of the genome. Finally, it is apparent that dominant lethality is an incompletely understood complex.

SUMMARY

1. The frequencies of X-ray-induced dominant and recessive lethal mutations in Habrobracon oocytes are unchanged by subsequent fertilization with unirradiated sperm.

2. A significant proportion of the dominant lethal effect induced at metaphase I is expressed at a later stage in diploids than in haploids.

3. When dominant lethals are induced at prophase I, the proportion expressed after hatching is the same in diploids as in haploids.

4. The dependence of delayed expression on the stage irradiated is consistent with the assumption that lethality caused by observable chromosome breakage can be delayed, whereas that due to other causes cannot. Chromosome breakage is apparently a more frequent cause of lethality after irradiation of oocytes in metaphase than in prophase.

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THE INDUCTION OF RECESSIVE LETHALS IN DROSOPHILA MELANOGASTER BY RADIATIONS OF DIFFERENT ION DENSITY[1]

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EARLY investigators in radiation genetics claimed that X-rays of different wave lengths, gamma rays, and beta particles of radium were equally effective in inducing sex-linked recessive mutations in *Drosophila melanogaster* and that densely ionizing radiations such as recoil protons from neutrons and alpha particles were less efficient than the less densely ionizing X, gamma, and beta radiations. This early work is described in reviews by ZIMMER and TIMOFÉEFF-RESSOVSKY (1942), CATCHESIDE (1948), and MULLER (1954). Later data, however, support opposite conclusions. MICKEY (1954) and IVES *et al.* (1954) found fast neutrons from the cyclotron and nuclear detonations to be 2–3.5 times as effective as X-rays in inducing recessive lethals in *D. melanogaster*, and KING (1954) reports that 90 kv X-rays are more efficient than beta particles from P^{32} bakelite plaques. The more recent data would, therefore, indicate that the relative biological effectiveness (RBE) of radiations of different ion densities increases with increasing ion density.

In the present study, a detailed comparison was made of the effects of fast neutrons, X-rays, and Co^{60} gamma-rays on the induction of recessive lethals in *D. melanogaster*. Results of this study also show that the RBE of different radiations increases with increasing ion density, and that the frequency of recessive lethals induced by X-rays increases more rapidly with increasing dose than expected on the basis of linearity.

MATERIALS AND METHODS

The interpretation of the results reported in this paper depend on accurate neutron dosimetry. A detailed description of the physical conditions for these neutron exposures has already been reported by SHEPPARD *et al.* (1956). All exposures were made in the biological facility of the Oak Ridge National Laboratory's 86-inch cyclotron. Bombardment of a beryllium target with 22 Mev protons produced neutrons whose energy ranged from that of thermal neutrons to about 3.5 Mev (only occasional neutrons had energy as high as 10 Mev), with a mean energy of ~1 Mev. Radiation from this reaction includes thermal neutrons and gamma rays as well as fast neutrons. The true neutron dose is derived through determination of the amount of radiation that each contaminating component contributes to the total dose. Thermal neutron contamination was negligible; the gamma-ray dose, at the exposure position in the biological facility, was about 7% of the total radiation dose. All exposures were made

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in thin (4 mm) lucite chambers placed in the center of the facility at the site of all dose measurements (SHEPPARD *et al.* 1956).

The gamma irradiations were made in a 300-curie Co^{60} source, similar to that already described by GHORMLEY and HOCHANADEL (1951), that emits 1.17 and 1.34 Mev gamma rays. Intensity of the radiation varied from 1600 r/min at the beginning of these experiments to approximately 1300 r/min at the end. The flies were placed in a gauze capsule and inserted in a lucite chamber that could be centered in the field of the source, after which the cobalt was lowered around the flies, producing a uniform radiation field.

All X-ray treatments were administered by a 250 kvp G.E. Maxitron machine operated at 250 kvp, 30 ma, with 3 mm of Al filter, producing an average intensity of about 225r/min at the target distance of 49 cm. Under these conditions, the half-value layer is 0.55 mm of Cu, which corresponds to an effective wave length of 0.20 A (KIRBY-SMITH and DANIELS 1953). Dose measurements and exposures were made inside the lucite tubes. Doses were measured with a 100r Victoreen ionization chamber before each X-ray exposure.

In those tests in which the RBE of fast neutrons and X-rays are compared to gamma rays in inducing sex-linked lethals, Oregon-R males, 2–4 days old, were exposed to four different doses of fast neutrons, seven of gamma rays, and nine of X-rays (see table 1). Control experiments were carried out simultaneously. The fre-

TABLE 1

The frequency of recessive lethals induced by fast neutrons, X-rays, and gamma-rays in Oregon-R males of Drosophila melanogaster

Radiation	Dose (r or rep)	No. of chromosomes tested	No. of lethals	Percentage of lethals ± 1 standard error
Fast neutrons	0	2225	3	0.14 ± 0.08
	299	2164	30	1.39 ± 0.25
	598	1873	34	1.82 ± 0.31
	1196	2124	74	3.48 ± 0.40
	1794	1595	92	5.77 ± 0.58
Gamma-rays	0	1875	1	0.053 ± 0.05
	1000	2799	61	2.18 ± 0.28
	2000	2764	117	4.23 ± 0.38
	3000	2322	123	5.30 ± 0.47
	4000	1801	137	7.61 ± 0.63
	4500	536	38	7.09 ± 1.11
	5000	971	93	9.58 ± 0.94
	6000	1948	253	12.99 ± 0.76
X-rays	0	3755	6	0.16 ± 0.07
	1000	2472	58	2.35 ± 0.31
	1500	1068	41	3.84 ± 0.59
	2000	2073	95	4.58 ± 0.46
	2500	1568	99	6.31 ± 0.59
	3000	2027	193	9.52 ± 0.65
	3500	912	90	9.87 ± 0.99
	4000	1503	186	12.38 ± 0.85
	5000	1635	194	11.87 ± 0.80
	6000	1395	191	13.69 ± 0.92

quency of sex-linked recessive lethals was determined by the Muller-5 technique (SPENCER and STERN 1948) or a modification of this method (EDINGTON and PARKER 1954). The criteria used for classifying recessive lethals were essentially those described by BAKER and EDINGTON (1952). In all radiation and control experiments, the parents were mass-mated, transferred to fresh cultures every two days for six days, and then discarded.

For the comparison of the frequency of recessive lethals induced in a ring and a rod chromosome, the ring-X, X^c, and an acrocentric (called "rod-X" chromosome in this paper), *In(1)EN*, which was derived from X^c (see NOVITSKI 1949), were used. Seven groups of males of the constitution X^c, *y/sc*^{8} *.Y* or *In(1)EN*, *y*/Y, 2–4 days old, were exposed to different doses of X-rays. At the same time, control experiments were run for both chromosomes. In this series, the X^c and *In(1)EN* males were mated to *In(1) dl-49, y w B* virgin females, and the frequency of recessive lethals determined as in the Muller-5 technique.

RESULTS

In order to measure accurately the RBE of radiations of different ion densities in inducing recessive lethals, the frequency of recessive lethals induced by each radiation at several dose levels was determined. Results of each experiment were subjected to chi-square tests for homogeneity; the variation observed in all tests was that expected from random sampling of a binomial population. Since no heterogeneity was observed at any dose for any radiation, the values reported at all doses (table 1) are those calculated from the pooled data.

The establishment of the binomial behavior of the recessive lethal frequencies indicated that weighted least-squares fits to the experimental points would provide the most accurate comparisons among the RBE's for the different radiations. Since the frequency-dose relation was assumed to be linear, a linear regression was calculated for each experiment. It then seemed advisable to test the experimental data for departure from linearity. Since the residual sum of squares in the weighted least-squares fits is distributed approximately as chi-square, this statistic was used to test for linearity. In Oregon-R males, the number of sex-linked recessive lethals induced by fast neutrons and gamma-rays did not depart significantly from linearity, with dose, but a significant departure was found for X-rays ($\chi^2 = 11.651$, d.f. $= 5$, $P =$ 0.02–0.05). Recessive lethals induced by X-rays in the *In(1)EN* chromosome (table 2) also deviated significantly from linearity ($\chi^2 = 17.338$, d.f. $= 6$, $P = <0.01$). Thus the likelihood that the nonlinearity observed in the Oregon-R data was spurious is greatly diminished. Nevertheless a second dose-curve experiment was carried out with the Oregon-R strain to check this observation. When the data were analyzed alone, it was found that they fitted a quadratic function ($\chi^2 = 6.1017$; d.f. $= 5$; $P = 0.269$) and less well a linear function ($\chi^2 = 10.2966$; d.f. $= 6$; $P = 0.117$). However, when the Oregon-R data were pooled, after homogeneity at each dose was proved, it was found that the pooled data deviated significantly from linearity ($\chi^2 = 15.0712$; d.f. $= 6$; $P = 0.019$). The frequencies of recessive lethals induced by X-rays at 4000, 5000, and 6000r were essentially the same. This plateau could be caused by the increasing frequency of induced dominant lethals, which could be

TABLE 2

The frequency of recessive lethals induced by X-rays in ring-X and rod-X chromosomes

Rod-X				Ring-X			
Dose (r)	No. of chromosomes tested	No. of lethals	Percentage of lethals ± 1 standard error	Dose (r)	No. of chromosomes tested	No. of lethals	Percentage of lethals ± 1 standard error
0	2643	11	0.42 ± 0.13	0	2335	6	0.26 ± 0.96
250	2461	27	1.10 ± 0.21	250	2111	20	0.95 ± 0.21
500	1898	17	0.90 ± 0.22	500	1619	26	1.61 ± 0.31
750	2179	41	1.88 ± 0.29	750	2054	31	1.51 ± 0.27
1000	1236	27	2.19 ± 0.42	1000	1120	32	2.86 ± 0.50
2000	1113	53	4.76 ± 0.64	2000	974	41	4.21 ± 0.64
3000	743	74	9.96 ± 1.10	3000	723	61	8.44 ± 1.03
4000	654	84	12.84 ± 1.31	4000	283	30	10.60 ± 1.83

correlated with induced recessive lethals and thus siphon off more and more recessive lethals at the higher doses. Since no easily defined function can be fitted to all these X-ray points, no attempt was made to carry out weighted fits that included doses above 4000r.

Upon discovery of the departures from linearity, the data up to and including 4000r for both experiments were refitted to quadratic equations yielding $y = 0.1612 + 1.7756\,D + 0.3302\,D^2$ for the Oregon-R results and $y = 0.4578 + 1.2789\,D + 0.4470\,D^2$ for *In(1)EN* results, where y is the predicted percentage of recessive lethals and D is the dose in kiloroentgens. Recomputation of the chi-squares for the quadratic fits showed that the inclusion of the D^2 term reduced the chi-square to levels that were very close to those expected ($\chi^2 = 4.5924$, d.f. = 5, $P = 0.46$) for Oregon-R and ($\chi^2 = 4.677$, d.f. = 5, $P = 0.46$) for *In(1)EN*. Also, the coefficient of the quadratic term was significantly different from zero ($P < 0.01$) for both equations. On the other hand, the frequency of recessive lethals induced by X-rays in the X^e chromosome (fig. 2) did not depart significantly from linearity; but for comparison with the *In(1)EN* chromosome, a quadratic equation was fitted to these data with the result, $y = 0.2983 + 1.9105\,D + 0.1798\,D^2$. The coefficient of the quadratic term in this equation did not differ significantly from zero.

In figure 1 the weighted linear regressions for fast neutrons and gamma rays and the weighted quadratic regression for X-rays are plotted. It can be seen that no dose-independent comparison of the RBE can be made between X-rays and either of the other two radiations. Since accurate measurements of the neutron effect requires correction of the data for gamma-ray contamination, a linearity term that accounted for the 7% contamination was used in a simultaneous fitting of the data for the neutron and gamma-ray experiments. The method used is similar to one used by RUSSELL *et al.* (1954) in a somewhat analogous situation. The neutron slope, corrected in this manner, is shown as the dashed line in figure 1.

A comparison of the slopes of the regressions of recessive lethals on dose for fast neutrons (3.15×10^{-3}) and gamma-rays (1.94×10^{-3}) gives a RBE of 1.62. Since, however, the X-ray data depart from linearity, the RBE of X-rays to gamma-rays is a function of dose and increases from 1.09 at 1000r to 1.60 at 4000r.

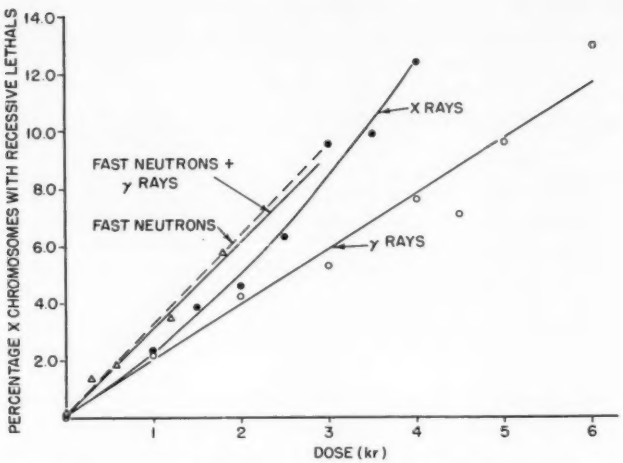

FIGURE 1.—The percentage of X chromosomes with recessive lethals induced by fast neutrons X-rays, and γ-rays in Oregon-R males of *Drosophila melanogaster*.

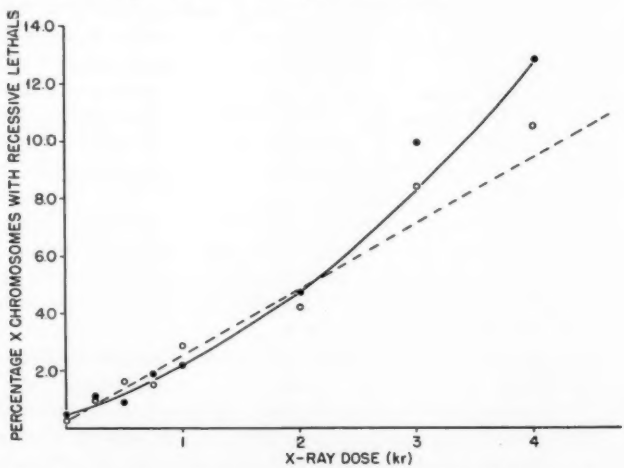

FIGURE 2.—The percentage of X chromosomes with recessive lethals induced by X-rays in rod-X (solid circles) and ring-X chromosomes (hollow circles).

The significant departure from linearity of recessive lethals induced by X-rays in the *In(1)EN* prevented comparison, by a simple STUDENT's *t* test, with the frequency of recessive lethals induced in the ring. Instead, the difference between X^c and *In(1)EN* was evaluated by the generalized T^2 test (RAO 1952), which amounts to an over-all comparison of the two quadratic dose-response curves. The dose-response

curves for the *In(1)EN* and X^c chromosomes were significantly different ($T^2 = 18.11$, $P = 0.041$).

DISCUSSION

It is evident from the results reported here that the determination of the RBE for sex-linked recessive lethals induced by radiations of different ion-density in *Drosophila melanogaster* depends on the kind of radiation and on the dose. For the dose range studied in these experiments, the RBE increased with increasing ion density, but with increasing dose the effect of X-rays becomes much greater than that from an equal dose of gamma-rays and approaches the effectiveness of the more densely ionizing neutrons. Hence, a dose-independent estimate can be made of the RBE for only fast neutrons as compared to gamma-rays, at least up to 6000r. In any comparison that involves X-rays, the RBE is meaningless.

The accuracy of the neutron exposures in these experiments may be checked by comparison of the present data with the corrected values for the recessive lethal data of MICKEY (1954), who has kindly consented to its incorporation in this paper. Both neutron exposures were made in the Oak Ridge National Laboratory 86-inch cyclotron (one year apart) and were dependent on the physical and statistical applications discussed by SHEPPARD *et al.* (1956). After MICKEY's results were published, a comprehensive statistical analysis of all the physical measurements was carried out and appropriate corrections were made for all biological exposures. The analysis showed that the slope of the regression for recessive lethals induced by fast neutrons (uncorrected for gamma contamination) was 3.11×10^{-3} for MICKEY's experiments instead of 6×10^{-3} as reported. This corrected value is in close agreement with the slope (3.02×10^{-3}) of the regression for fast neutrons shown in figure 1.

It should be noted that, in view of the nonlinear increase of recessive lethals induced by X-rays one would have expected a nonlinear increase with gamma-rays, over the gamma-dose-range studied. This was not observed, for reasons as yet unknown; however, experiments are in progress to confirm or refute this difference.

It is well known that one-hit genetic effects induced by all types of radiations increase linearly with dose and that two-hit events induced by densely ionizing radiations also increase linearly with increasing dose. However, gross structural changes induced by less densely ionizing radiations increase more rapidly than the first power of the dose. This difference is explained on the assumption that a single recoil proton can produce both breakage events, whereas the two breaks are induced independently by the less densely ionizing radiations.

The observed departure from linearity of X-ray-induced recessive lethals may be explained only through a consideration of the genetic effects associated with two independent events that could behave as recessive lethals. Any one or a combination of all of the following genetic effects could contribute the two-hit component to the total recessive lethal frequency: (1) two semilethals, which together on the same chromosome behave as a single recessive lethal; (2) gross interstitial deficiencies; and (3) "position effect" lethals, which are dependent on otherwise viable gross chromosome rearrangements for their expression.

A comparison of the frequencies of recessive lethals induced by X-rays in the ring-X

and the rod-X chromosomes shows a significant departure from linearity in the frequency-dose relation for the rod but not for the ring. This difference in response of the ring and the rod to radiation may be explained on the basis of a selective elimination of some of the breakage events induced in the ring chromosome. It has been suggested that simple breakage and then torsional restitution in the ring will produce either loss of the ring or dominant lethality to the potential zygote (MULLER 1940; 1940 CATCHESIDE and LEA 1945). Therefore, on the assumption that semilethals are correlated with breakage, genetic effects (1) and (2) could be eliminated in the ring more often than in the rod. It is also known that all two-hit translocations involving the ring chromosome, and possibly some ring-X chromosome inversions, are lost owing to the production of dicentric chromosomes (CATCHESIDE and LEA 1945). Consequently, more lethals associated with rearrangement would be eliminated in the ring than in the rod. Thus the total frequency of recessive lethals induced by X-rays in the rod-X and ring-X chromosomes might be expected to depart from linearity at the higher doses, but in a much smaller degree in the ring than in the rod.

SUMMARY

An investigation of the relative biological effectiveness of fast neutrons, X-rays, and Co^{60} gamma-rays in inducing sex-linked recessive lethals shows that fast neutrons are 1.62 times as effective as gamma-rays and that any RBE comparison involving X-rays is dependent on the dose of X-rays used. It is also shown that the frequency of recessive lethals induced by X-rays departs significantly from linearity as the radiation dose increases, but that neither the fast neutron nor the gamma-ray dose curves depart significantly from linearity over the dose ranges studied. It was also demonstrated that the frequency of recessive lethals induced by X-rays in the *In(1)EN* and X^c chromosomes were significantly different and that the difference may be attributed to loss of an increasing number of lethals associated with rearrangements and other possible two-hit events in the ring at the higher doses.

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THE MODIFIED DIALLEL TABLE WITH PARTIAL INBREEDING AND INTERACTIONS WITH ENVIRONMENT[1]

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THE reasons for considering the covariances of relatives are firstly that they will be of interest *per se* in indicating the regression of offspring on parent etc., and secondly that they lead to the estimation of genetic parameters which are useful as a description of a genetic population and in the formulation of breeding procedures.

Various methods of obtaining theoretical covariances between relatives have been developed, each with a specific set of assumptions. The problem was first considered for random mating populations in equilibrium by FISHER (1918). Correlations were obtained for parent-offspring, full-sib, half-sib, uncle-nephew, cousin, and double-first-cousin relationships for any degree of dominance. The general solution included an arbitrary number of alleles with two-factor epistacy considered for parent-offspring and full-sib correlations. WRIGHT (1921) proposed the method of path coefficients for the study of correlations between relatives with respect to genes which are strictly additive in their effects.

The analysis of diallel crosses presented by HAYMAN (1954a, 1954b) considered an arbitrary degree of dominance for the case where the parents in the diallel table are completely homozygous. Only two alleles per locus were considered and the assumption of no epistacy was imposed. JINKS (1954) applied this method to a study of height, flowering time, and leaf length in *Nicotiana rustica* to estimate the average degree of dominance. The regression of array covariance on variance was plotted to obtain evidence of non-additive gene effects. Deviations from unit slope indicated non-allelic gene interaction might be responsible for the observed heterosis in some F_1 hybrids. Further applications of this method to actual data were given by JINKS (1955). DICKINSON and JINKS (1956) extended the methods of HAYMAN and JINKS to include inbreeding of the parental material in the diallel table.

COCKERHAM (1954) has considered the covariances between relatives under random mating of an inbred population for the case of two alleles per locus. These covariances are expressible in terms of the components of genotypic variance of the random mating population which was the origin of the inbred population.

GRIFFING (1956) considered diallel crosses of homozygous individuals obtained from a random mating population and presented the genetic parameters in terms of additive, dominance, and epistatic effects for an arbitrary number of loci with arbitrary alleles. The term "modified diallel" was suggested to designate the diallel table where the parents are not included. The variances of specific and general combining ability were given in terms of additive, dominance, and epistatic variances.

ALLARD (1956) has given some procedures for evaluating genotype-environment

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interactions in the diallel table. His procedures are a development of the work by HAYMAN and JINKS.

KEMPTHORNE (1956a) removed the assumptions of no epistacy and of only two alleles per locus as given by HAYMAN (1954b) and presented the analysis of diallel crosses for arbitrary alleles, arbitrary loci, and arbitrary epistacy. The parents in the diallel table were homozygous lines obtained by inbreeding without selection from a random mating population. Estimates of the mean and variance of both the random mating and homozygous populations, and the parent-offspring covariance were obtained from the variances and covariances of the diallel table. Evidence was given to show that previous methods of analysis of diallel crosses are of questionable validity.

In the present discussion the previous methods will be extended to include the case of arbitrary inbreeding for the parents in the modified diallel table and the analysis of variance is presented for obtaining estimates of the interactions of genotypic components of variance with environments as represented by locations and years.

COVARIANCES BETWEEN RELATIVES

As a method of determining what happens to the additive, dominance, and epistatic deviations under a random mating system when individuals are inbred and then crossed in all possible crosses, the covariance of full-sibs, $Cov(FS)$, and the covariance of half-sibs, $Cov(HS)$, must be obtained for an arbitrary degree of inbreeding assuming any number of loci with an arbitrary number of alleles.

If two individuals are mated which are more closely related than the average of the population, the resulting progeny are inbred. The two genes at a locus of an individual can be alike because they descend from one gene or because of random sampling from a population. The probability of two genes being identical by descent is F, the inbreeding coefficient, which varies in the range from zero in a random mating population to one in an inbred population. Inbreeding and gene frequency are completely independent, with inbreeding changing the association of the alleles in pairs while gene frequency is the proportion of each allele in the population. Under inbreeding the genotypic array for a single locus with arbitrary alleles is

$$F \sum_i p_i A_i A_i + (1 - F) \sum_{ij} p_i p_j A_i A_j .$$

The genotypic value of an individual y_{ijkl} considering two loci can be written as

$$\begin{aligned} y_{ijkl} = \mu &+ \alpha_i^1 + \alpha_j^1 + (\alpha^1\alpha^1)_{ij} + \alpha_k^2 + \alpha_l^2 \\ &+ (\alpha^2\alpha^2)_{kl} + (\alpha^1\alpha^2)_{ik} + (\alpha^1\alpha^2)_{jk} + (\alpha^1\alpha^2)_{il} \\ &+ (\alpha^1\alpha^2)_{jl} + (\alpha^1\alpha^2\alpha^2)_{ikl} + (\alpha^1\alpha^2\alpha^2)_{jkl} \\ &+ (\alpha^1\alpha^1\alpha^2)_{ijk} + (\alpha^1\alpha^1\alpha^2)_{ijl} + (\alpha^1\alpha^1\alpha^2\alpha^2)_{ijkl} \end{aligned} \tag{1}$$

where the superscripts indicate the locus (KEMPTHORNE 1956b, 1956c), and for example, α_i^1 is the average effect of the ith allele at locus 1, $(\alpha^2\alpha^2)_{kl}$ is the dominance effect at locus 2, $(\alpha^1\alpha^2)_{ik}$ is the interaction of the additive effect of the ith allele at

locus 1 with the additive effect of the kth allele at locus 2, and the other terms have similar meaning.

The detailed derivation of the covariances was given by MATZINGER (1956). Since it is quite lengthy and tedious it will be indicated only for the case of two loci, and the general results will be presented. Linkage is assumed to be absent.

Variance between family means

Consider a cross of $A_iA_jB_kB_l \times A_mA_nB_rB_s$ where A and B are two independent loci, and the subscripts indicate the allele at that locus. Let the frequencies of A_iA_j, B_kB_l, A_mA_n, and B_rB_s be designated as P_{ij}, P'_{kl}, P_{mn}, and P'_{rs} respectively, where for example

$$P_{ij} = (1 - F)p_ip_j + \delta_{ij}Fp_i$$

and

$$\delta_{ij} = 1 \text{ if } i = j$$
$$= 0 \text{ if } i \neq j.$$

The variance between family means is obtained as

$$\sum_{\substack{ijkl \\ mnrs}} P_{ij}P'_{kl}P_{mn}P'_{rs}[\{\tfrac{1}{2}(A_i + A_j)\tfrac{1}{2}(A_m + A_n)\}\{\tfrac{1}{2}(B_k + B_l)\tfrac{1}{2}(B_r + B_s)\}]^2$$

in which each genotype symbol is replaced by the corresponding genotypic value and μ is replaced by zero. To solve this expression the terms are written in the form of additive, dominance, and interaction effects of the type in equation (1).

After obtaining the simplification of this equation into components of genotypic variance as given by KEMPTHORNE (1954, 1955) and COCKERHAM (1954), the variance between family means can be written down and extended to the case of any number of loci. In the present case this variance is the covariance of full-sibs and is given by

$$\begin{aligned} Cov(FS) = &\left(\frac{1+F}{2}\right)\sigma^2_A + \left(\frac{1+F}{2}\right)^2\sigma^2_D + \left(\frac{1+F}{2}\right)^2\sigma^2_{AA} \\ &+ \left(\frac{1+F}{2}\right)^3\sigma^2_{AD} + \left(\frac{1+F}{2}\right)^4\sigma^2_{DD} + \left(\frac{1+F}{2}\right)^3\sigma^2_{AAA} \\ &+ \left(\frac{1+F}{2}\right)^4\sigma^2_{AAD} + \left(\frac{1+F}{2}\right)^5\sigma^2_{ADD} + \left(\frac{1+F}{2}\right)^6\sigma^2_{DDD} \\ &+ \text{etc.} \end{aligned} \tag{2}$$

Variance between sire means

Let the genotype of an arbitrary sire be $A_iA_jB_kB_l$ with frequency $P_{ij}P'_{kl}$. The dam gametic array is

$$\{\sum_r p_rA_r\}\{\sum_s p'_sB_s\}$$

and the sire gametic array is

$$\{\tfrac{1}{2}A_i + \tfrac{1}{2}A_j\}\{\tfrac{1}{2}B_k + \tfrac{1}{2}B_l\}.$$

The offspring array is therefore

$$\{\tfrac{1}{2}\sum_r p_r A_r A_i + \tfrac{1}{2}\sum_r p_r A_r A_j\}\{\tfrac{1}{2}\sum_s p'_s B_s B_k + \tfrac{1}{2}\sum_s p'_s B_s B_l\}.$$

Substitution in terms of the genotypic values of formula (1) and simplifying leads to the variance between sires for two loci which can easily be extended to arbitrary loci.

Since each sire is mated to dams obtained at random from a random mating population the variance of sire means is the covariance of half-sibs which is given by the equation

$$Cov(HS) = \left(\frac{1+F}{4}\right)\sigma^2_A + \left(\frac{1+F}{4}\right)^2\sigma^2_{AA} + \left(\frac{1+F}{4}\right)^3\sigma^2_{AAA} + \text{etc.} \qquad (3)$$

These values for $Cov(FS)$ and $Cov(HS)$ are equivalent to those obtained by COCKERHAM (1954) for the case of only two alleles per locus.

STRUCTURE OF MODIFIED DIALLEL TABLE

The experiment to be considered involves all possible crosses between p parents with a given degree of inbreeding, or if one assumes the absence of maternal effects, reciprocal crosses need not be obtained and one has $p(p-1)/2$ families. The analysis discussed below assumes there are no maternal effects.

The analysis of variance of diallel crosses was presented by YATES (1947) and in various other forms by other workers. A model used to analyze the variation of a single experiment is

$$y_{ijk} = \mu + g_i + g_j + s_{ij} + r_k + e_{ijk} \qquad (4)$$

where

$$i, j = 1, 2, \cdots, p \; i < j$$

$$k = 1, 2, \cdots, r$$

and y_{ijk} is the yield resulting from a cross of the ith line with the jth line grown in the kth replicate; μ is a common mean for all crosses in all replicates; g_i is a measure of the average effect of the ith line to all progeny; s_{ij} is a measure of the average effect of a cross of the ith line and the jth line; r_k is the average effect of all crosses grown in the kth replicate; and e_{ijk} is the experimental error associated with the y_{ijk} observation. The g_i components measure general combining ability, while the s_{ij}'s measure deviations from an additive genetic model, frequently referred to as specific combining ability. All variable components in the model (4) are assumed to have zero expectation, to be uncorrelated, and to have their particular variances.

ROJAS (1951) considered the analysis of variance for tests of hypotheses and estimation of variance components by least squares estimates. The expected mean squares were obtained by finding the expected value of the sums of squares in terms of the original model (4) and dividing by the degrees of freedom.

TABLE 1

Analysis of variance and expected mean squares of modified diallel crosses

Source	d.f.	S.S.	M.S.	E.M.S.*			
				σ^2	σ_a^2	σ_g^2	$\frac{1}{(r-1)}\sum_k r_k^3$
				$\sigma_e^2+\sigma_1^2$	$-\sigma_2^2$	$\sigma_2^2-2\sigma_3^2$	σ_3^2
Replicates	$r-1$	$R=\sum_k \frac{Y_{\cdot\cdot k}^2}{n}-C$	R'	1	0	0	n
g_i's	$p-1$	$G=\frac{1}{r(p-2)}\sum_i Y_{i\cdot\cdot}^2-\frac{2(p-1)C}{(p-2)}$	G'	1	r	$r(p-2)$	0
s_{ij}'s	$p(p-3)/2$	$S=\sum_{ij}\frac{Y_{ij\cdot}^2}{r}-C-G$	S'	1	r	0	0
Error	$(r-1)(n-1)$	$E=T-S-G-R$	E'	1	0	0	0
Total	$rn-1$	$T=\sum_{ijk} y_{ijk}^2-C$					

* Expectations obtained by ROJAS (1951) above; expectations in terms of concepts of present paper below.

The expected mean squares obtained by ROJAS in relation to variances of general and specific combining ability are presented in table 1. These variances were obtained for the original definitions of general and specific combining ability given by SPRAGUE and TATUM (1942). In the present study the expected mean squares are derived in terms of variances and covariances of relatives with respect to a random mating population by again taking the expectation of the sums of squares in table 1.

Consider a diallel table where a plot is the progeny of a mating of two parents with a known amount of inbreeding. Variances and covariances of plot totals are obtained for a constant number, f, plants per plot. The case of unequal numbers of plants per plot presents a slight complication and is given by KEMPTHORNE (1956c).

All of the individuals in a plot have the same sire and dam so the plot total contains f plants which are full-sibs. The expected value of a plot total is $f\mu_G$ where μ_G is the mean of the genotypic portion of the individuals. Then

$$E(\textit{plot total})^2 = f^2\mu_G^2 + f\sigma_G^2 + f(f-1)Cov(FS)$$

where σ_G^2 is the genotypic variance. The genotypic variance of a plot total is obtained by the usual method as

$$\begin{aligned} V(\textit{plot total}) &= E\,(\textit{plot total})^2 - \{E(\textit{plot total})\}^2 \\ &= f\sigma_G^2 + f(f-1)Cov(FS) = \sigma_1^2. \end{aligned} \tag{5}$$

It is assumed that environment within the experiment is additive with variance σ_e^2, so that the total variance of a plot total equals $\sigma_e^2 + \sigma_1^2$.

Next consider the covariance of two plots, both with the same sire and dam, but in different replicates. If the parents are not homozygous the individuals in one replicate are not a true duplication of the genotypic value of individuals in the same entry in another replicate. In fact, the individuals are full-sibs. With f plants per plot, the covariance between two plots with the same parents, in different replicates, is

$$f^2\,Cov(FS) = \sigma_2^2. \tag{6}$$

The covariance between plots with one parent in common is a function of the half-sib covariance. With f individuals per plot this covariance will be

$$f^2\, Cov(HS) = \sigma_3^2. \tag{7}$$

The expected mean squares are derived in terms of σ_1^2, σ_2^2, and σ_3^2. The mean is coded as zero so it will not enter into the derivation. Replicates are orthogonal to crosses and their effects can be ignored in most of the computations which follow. Terms involving both the mean and replications will cancel out if carried along. The experimental error will contribute σ_e^2 to each expected mean square and is omitted in the following derivations.

The $Y_{i..}$ term, a sire total, contains $r(p-1)$ plots, and $Y_{i..}^2$ will involve $r(p-1)$ σ_1^2 terms. Each of the $(p-1)$ plot totals is a full-sib to $r(r-1)$ plot totals in other replicates. Each of $(p-1)$ plot totals in one replication is a half-sib with $(p-2)$ plots in r replicates, over the total of r replicates. Then

$$E(Y_{i..}^2) = r(p-1)\sigma_1^2 + r(r-1)(p-1)\sigma_2^2 + r^2(p-1)(p-2)\sigma_3^2.$$

The grand total, $Y...$, contains a total of rn plots. The square of this term will contain rn terms of σ_1^2. The full-sib comparisons will be $p/2$ times those for $Y_{i..}$ since all of the dams are also included. Each of n plot totals in one replicate is a half-sib of $2(p-2)$ plots over r replicates, and

$$E(Y_{...}^2) = rn\sigma_1^2 + r(r-1)n\sigma_2^2 + 2r^2(p-2)n\sigma_3^2.$$

Each $Y_{ij.}$ term contains r plots, so $Y_{ij.}^2$ has r terms of σ_1^2. The full-sib covariances will enter between the replicates $r(r-1)$ times, and there are no half-sib covariances. Then

$$E(Y_{ij.}^2) = r\sigma_1^2 + r(r-1)\sigma_2^2.$$

A total of n plots are contained in each $Y_{..k}$ term. In $Y_{..k}^2$ there will be n terms of σ_1^2, and no terms in σ_2^2 because there are no comparisons between replicates. Each of n plot totals will involve half-sib covariances with $2(p-2)$ plots resulting in

$$E(Y_{..k}^2) = n\sigma_1^2 + 2n(p-2)\sigma_3^2 + n^2r_k^2.$$

The genotypic part of the expected values of the sums of squares in table 1 are as follows:

$$\begin{aligned}
E(C) &= \sigma_1^2 + (r-1)\sigma_2^2 + 2r(p-2)\sigma_3^2 \\
E(R) &= (r-1)\sigma_1^2 - (r-1)\sigma_2^2 + n\sum_k r_k^2 \\
E(G) &= (p-1)\sigma_1^2 + (p-1)(r-1)\sigma_2^2 + r(p-1)(p-4)\sigma_3^2 \\
E(S) &= \tfrac{1}{2}p(p-3)\sigma_1^2 + \tfrac{1}{2}(r-1)p(p-3)\sigma_2^2 - rp(p-3)\sigma_3^2 \\
E(E) &= (r-1)(n-1)\sigma_1^2 - (r-1)(n-1)\sigma_2^2 \\
E(T) &= (rn-1)\sigma_1^2 - (r-1)\sigma_2^2 - 2r(p-2)\sigma_3^2.
\end{aligned}$$

Dividing the expected sums of squares by degrees of freedom gives the expected mean squares in table 1 after slight rearrangement. Each component of variation also contains a term for plot error (σ_e^2) as indicated in table 1.

The similarity between the expected mean squares of ROJAS and those obtained in this paper is very evident in table 1. After substitution for σ_1^2, σ_2^2, and σ_3^2 from equations (5), (6), and (7) the equality is

$$\begin{aligned} \sigma^2 &= \sigma_e^2 + f\{\sigma_G^2 - Cov(FS)\} \\ \sigma_s^2 &= f^2\{Cov(FS) - 2Cov(HS)\} \end{aligned} \tag{8}$$

$$\sigma_g^2 = f^2\, Cov(HS). \tag{9}$$

Estimates of these variance components are obtained by equating the expected mean squares to the corresponding observed mean squares and solving for the desired component as follows:

$$\widehat{\sigma_g^2} = \frac{G' - S'}{r(p-2)}$$

$$\widehat{\sigma_s^2} = \frac{S' - E'}{r}$$

$$\widehat{\sigma^2} = E'.$$

$Cov(FS)$ and $Cov(HS)$ can be obtained from these estimates as

$$\widehat{Cov(HS)} = \frac{\widehat{\sigma_g^2}}{f^2}$$

$$\widehat{Cov(FS)} = \frac{\widehat{\sigma_s^2} + \widehat{2\sigma_g^2}}{f^2}.$$

From the theoretical derivation of $Cov(FS)$ and $Cov(HS)$ in equations (2) and (3), estimates of the components of genotypic variance may be obtained. The values of σ_g^2 and σ_s^2 may be expressed in terms of additive, dominance, and epistatic variances to observe the type of epistatic deviations which contribute to each of these variances. The estimates obtained from equations (8) and (9) and equations (1) and (2) are

$$\sigma_g^2 = f^2\left\{\left(\frac{1+F}{4}\right)\sigma_A^2 + \left(\frac{1+F}{4}\right)^2 \sigma_{AA}^2 + \text{etc.}\right\}$$

$$\sigma_s^2 = f^2\left\{\left(\frac{1+F}{2}\right)^2\sigma_D^2 + \frac{(1+F)^2}{8}\,\sigma_{AAA}^2 + \left(\frac{1+F}{2}\right)^3\sigma_{AD}^2 + \left(\frac{1+F}{2}\right)^4\sigma_{DD}^2 + \text{etc.}\right\}.$$

Estimates of σ_A^2, σ_D^2, and the epistatic components can be obtained if a series of diallel cross experiments is made with different levels of inbreeding. From the diallel crosses of parents with a single level of inbreeding, estimates of σ_A^2 and σ_D^2 can be

obtained only under the assumption of no epistacy. Estimates of σ_A^2, σ_D^2, and σ_G^2 are then obtained as

$$\widehat{\sigma_A^2} = \frac{4\widehat{Cov(HS)}}{1 + F}$$

$$\widehat{\sigma_D^2} = \frac{4\{\widehat{Cov(FS)} - 2\widehat{Cov(HS)}\}}{(1 + F)^2}$$

and

$$\widehat{\sigma_G^2} = \widehat{\sigma_A^2} + \widehat{\sigma_D^2}.$$

For the case of $F = 1$, GRIFFING (1956) showed that the following relationship existed with σ_g^2 and σ_s^2 defined in terms of plot means:

$$\sigma_G^2 = 2\sigma_g^2 + \sigma_s^2.$$

It can be seen that this result is applicable only to the case of $F = 1$. From the expectations in the present paper the general relationship would be

$$2\sigma_g^2 + \sigma_s^2 = Cov(FS)$$

and only for the specific case of $F = 1$ would it be the case that

$$Cov(FS) = \sigma_G^2.$$

The value of F to be used in the formulae depends upon the source of the material used as parents in the diallel table. Normally diallel crosses are made between families and not single individuals. Families which arise from inbreeding without selection in a random mating population in equilibrium will reconstitute the original random mating population when crossed, because the gametic array, and hence the gene frequency, will not change with different levels of inbreeding. The appropriate procedure is to obtain the inbred population by whatever means are most suitable and then to choose individuals at random from this population to serve as parents. Each parent is then selfed to give a sufficient number of plants to enable the crosses to be made. The appropriate F value is that holding before the aforementioned multiplication. In the case of inbred lines in animals one could obtain sufficient individuals by full-sibbing.

ANALYSIS OF EXPERIMENTS COMBINED OVER LOCATIONS AND YEARS

Of major interest in assessing the role of the different types of gene effect and in a practical breeding program is the estimation of the components of genotypic variance over locations and years, and the components of variance due to possible interactions of genotypes with each. An extension of the model for a single experiment to include locations and years is

$$\begin{aligned} y_{ijktq} = \mu &+ g_i + g_j + s_{ij} + d_k + y_t + (gd)_{ik} + (gd)_{jk} \\ &+ (sd)_{ijk} + (gy)_{it} + (gy)_{jt} + (sy)_{ijt} \\ &+ (dy)_{kt} + (gdy)_{ikt} + (gdy)_{jkt} + (sdy)_{ijkt} \\ &+ r_{ktq} + e_{ijktq} \end{aligned}$$

TABLE 2

Expected mean squares for components of genotypic variance in experiments repeated over locations and years

Source	E.M.S.*
g_i's	$\sigma_4^2 + r\sigma_{5dy}^2 + br\sigma_{5y}^2 + cr\sigma_{5d}^2 + bcr\sigma_5^2 + r(p-2)\sigma_{3dy}^2 + br(p-2)\sigma_{3y}^2 + cr(p-2)\sigma_{3d}^2 + bcr(p-2)\sigma_3^2$
s_{ij}'s	$\sigma_4^2 + r\sigma_{5dy}^2 + br\sigma_{5y}^2 + cr\sigma_{5d}^2 + bcr\sigma_5^2$
$(gd)_{ik}$'s	$\sigma_4^2 + r\sigma_{5dy}^2 + cr\sigma_{5d}^2 + r(p-2)\sigma_{3dy}^2 + cr(p-2)\sigma_{3d}^2$
$(sd)_{ijk}$'s	$\sigma_4^2 + r\sigma_{5dy}^2 + cr\sigma_{5d}^2$
$(gy)_{it}$'s	$\sigma_4^2 + r\sigma_{5dy}^2 + br\sigma_{5y}^2 + r(p-2)\sigma_{3dy}^2 + br(p-2)\sigma_{3y}^2$
$(sy)_{ijt}$'s	$\sigma_4^2 + r\sigma_{5dy}^2 + br\sigma_{5y}^2$
$(gdy)_{ikt}$'s	$\sigma_4^2 + r\sigma_{5dy}^2 + r(p-2)\sigma_{3dy}^2$
$(sdy)_{ijkt}$'s	$\sigma_4^2 + r\sigma_{5dy}^2$
Error	σ_4^2

* Expected mean squares coded as

$$\sigma_4^2 = \sigma_e^2 + \sigma_1^2 - \sigma_2^2$$
$$\sigma_{5dy}^2 = \sigma_{2dy}^2 - 2\sigma_{3dy}^2$$
$$\sigma_{5y}^2 = \sigma_{2y}^2 - 2\sigma_{3y}^2$$
$$\sigma_{5d}^2 = \sigma_{2d}^2 - 2\sigma_{3d}^2$$
$$\sigma_5^2 = \sigma_2^2 - 2\sigma_3^2$$

where y_{ijktq} is the yield of a cross of the ith and jth lines in the qth replicate in the kth location in the tth year; μ is a common mean for all crosses in r replicates, b locations, and c years; g_i is a measure of the average effect of the ith line to all progeny; s_{ij} is a measure of the average effect of a cross of the ith line with the jth line; d_k is the average effect of the kth location; y_t is the average effect of the tth year; $(gd)_{ik}$ is the average interaction of the ith line with the kth location; and the other interactions have the appropriate meaning designated by the subscripts. In all cases the averages are over the populations of other factors. All parameters in the model except μ and r_{ktq} are considered random with expectation zero and zero correlation.

The components of variance of general and specific combining ability over locations and years were presented by ROJAS and SPRAGUE (1952). The expected mean squares in terms of genotypic components can be extended over locations and years, because crosses and hence comparisons of the g_i's or s_{ij}'s are orthogonal to locations and years. These are presented in table 2.

The values of σ_1^2, σ_2^2, and σ_3^2 have the same meanings as in the individual experiments except that they are defined as variances or covariances of averages over the population of environments which is assumed to be indefinitely large. The addition of d or y to these terms indicates the corresponding interactions. For example, σ_{2dy}^2 is the 3-way interaction of locations and years with a pseudo-factor x, say, which gives rise to the variance σ_2^2.

Because of the relationships between the expected mean squares in table 2 and those of ROJAS and SPRAGUE, estimates of $Cov(HS)$, $Cov(FS)$, and all interaction terms can be obtained from the following relationships:

$$\sigma_g^2 = \sigma_2^2 = f^2Cov(HS)$$
$$\sigma_s^2 = \sigma_5^2 = f^2\{Cov(FS) - 2Cov(HS)\}$$
$$\sigma_{gd}^2 = \sigma_{2d}^2 = f^2Cov(HS)_d$$
$$\sigma_{sd}^2 = \sigma_{5d}^2 = f^2\{Cov(FS)_d - 2Cov(HS)_d\}$$
$$\sigma_{gy}^2 = \sigma_{3y}^2 = f^2Cov(HS)_y$$
$$\sigma_{sy}^2 = \sigma_{5y}^2 = f^2\{Cov(FS)_y - 2Cov(HS)_y\}$$
$$\sigma_{gdy}^2 = \sigma_{3dy}^2 = f^2Cov(HS)_{dy}$$
$$\sigma_{sdy}^2 = \sigma_{5dy}^2 = f^2\{Cov(FS)_{dy} - 2Cov(HS)_{dy}\}$$
$$\sigma^2 = \sigma_4^2 = \sigma_e^2 + f\{\sigma_G^2 - Cov(FS)\}$$

The term $Cov(HS)_d$, for example, is the component for the interaction of the likeness of half-sibs with locations. Estimates of the components of variance are obtained by equating the expected mean squares to the observed mean squares and solving for the desired components.

Obtaining estimates of additive, dominance, and epistatic components and interactions with locations and years depends upon the number of levels of inbreeding. For example, for modified diallel crosses of one level of inbreeding in experiments repeated over locations and years only additive and dominance variances and corresponding interactions can be estimated assuming no epistacy by the following equations:

$$\sigma_A^2 = \frac{4Cov(HS)}{(1+F)}$$
$$\sigma_D^2 = \frac{4\{Cov(FS) - 2Cov(HS)\}}{(1+F)^2}$$
$$\sigma_{Ay}^2 = \frac{4Cov(HS)_y}{(1+F)}$$
$$\sigma_{Dy}^2 = \frac{4\{Cov(FS)_y - 2Cov(HS)_y\}}{(1+F)^2}$$
$$\sigma_{Ad}^2 = \frac{4Cov(HS)_d}{(1+F)}$$
$$\sigma_{Dd}^2 = \frac{4\{Cov(FS)_d - 2Cov(HS)_d\}}{(1+F)^2}$$
$$\sigma_{Ady}^2 = \frac{4Cov(HS)_{dy}}{(1+F)}$$
$$\sigma_{Ddy}^2 = \frac{4\{Cov(FS)_{dy} - 2Cov(HS)_{dy}\}}{(1+F)^2}$$
$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2$$
$$\sigma_{Gy}^2 = \sigma_{Ay}^2 + \sigma_{Dy}^2$$
$$\sigma_{Gd}^2 = \sigma_{Ad}^2 + \sigma_{Dd}^2$$
$$\sigma_{Gdy}^2 = \sigma_{Ady}^2 + \sigma_{Ddy}^2.$$

If the original population had two equally frequent alleles at each variable locus, one could estimate the average squared degree of dominance by $2\hat{\sigma}^2_D/\hat{\sigma}^2_A$.

EXTENSION TO OTHER EXPERIMENTS

With more complete data estimates can be obtained on epistatic parameters and all possible interactions with environment. Estimates from diallel crosses within a given level of inbreeding of the parents can be combined to give information on these components.

The present discussion has been for the analysis of plot totals. Additional information can be obtained from the modified diallel table if individual plant data are obtained. The procedure then is an analysis of variance between and within plots. The expectation of the mean square within plots is

$$\sigma^2_p + \{\sigma^2_G - Cov(FS)\}$$

where σ^2_p is the error attributed to each individual within the plot.

LINKAGE BIAS

In this discussion the genotypic frequencies were assumed to be in linkage equilibrium. However, even if the population is in linkage equilibrium these linkages will affect the covariances, as shown by COCKERHAM (1956) for the case when F equals zero. Assuming no position effects, the covariances between relatives where one is an ancestor of the other are not affected by linkage. However, for full-sibs and half-sibs, linkage causes a bias in the epistatic components of variance, but not in the additive or dominance components. A formulation of the general effects of linkage is one of the outstanding problems in the whole area of quantitative inheritance.

SUMMARY

The theoretical covariances between full-sibs and half-sibs under random mating in an arbitrarily inbred population are presented for the case of arbitrary numbers of alleles and loci, and arbitrary epistacy.

The results are applied to the modified diallel cross table in which the parents are of the same known degree of inbreeding. The theoretical relationships between general combining ability and specific combining ability variances, and the components of genotypic variance in the random mating population are obtained.

The procedures for analysis and interpretation of modified diallel crosses replicated over locations and years are presented.

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GENETIC STUDIES OF CELLULAR ANTIGENS IN THE CHICKEN[1]

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LINKAGE involving a gene for a cellular antigen was first reported by SAWIN, GRIFFITH and STUART (1944) for brachydactyly and the A agglutinogen in rabbits. In chickens a recombination frequency of 39.8% was reported for the crest gene and the gene for the cellular antigen X3 (BRILES 1951). The latter report followed closely the investigations of BRILES, MCGIBBON and IRWIN (1950) which clearly demonstrated the existence of two loci with multiple alleles for cellular antigens in the chicken. The evidence for two linkage groups of genes for erythrocyte antigens in the chicken will be presented in this paper as well as data for blood group genes whose linkage relations are not known.

METHODS AND MATERIALS

In general, the procedures used in this study are similar to those of BRILES, MCGIBBON and IRWIN (1950). Reagents were obtained by differential absorption of chicken isoantisera and antisera obtained from rabbits. The titers of the reagents used in this study ranged from 1:4 to 1:64. Agglutination tests were used to detect the presence of antigen or antibody specificities following pertinent absorptions. In these tests mixtures of red blood cells and antiserum were incubated at 31°–32°C for two hours, at which time the tubes were examined for evidence of agglutination. The tubes were then kept in the refrigerator at 4°C for 18 to 24 hours and again examined. The effect of the additional period of incubation at 4°C is to increase slightly the amount of agglutination, possibly to the next or second higher score (defined below). Incubation for four hours at 31°–32°C is comparable to the additional incubation at 4°C in that the change is toward increased agglutination. Very rarely (3 out of 499 mixtures of cells and reagent) was agglutination present at four hours of incubation when it was not observed at two hours. Incubation for four hours has been used in some agglutination tests reported in this paper and is now standard practice.

Tubes in which agglutination could not be observed or in which weak agglutination occurred were rechecked microscopically. The method of scoring agglutination is similar to the one used by BRILES *et al.* (1950). The absence of a reaction is scored 0 and a score of 4 is recorded for maximum agglutination, i.e. a solid clump of all cells in the tube. Scores between zero and four are recorded for degrees of reaction less than maximum. A score of ± is used to indicate a questionable reaction which was checked microscopically. Reagents were designated by the prefix, "anti", e.g.

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anti-M. Antigenic substances and their causative genes were designated by the same letter with subscript numbers used to depict each allele and its antigenic product. Reagents which have not been used in progeny tests or which are in the process of being tested have been designated solely by number in the sequence of discovery. The rationale involved in the analysis of anti-sera from which reagents were obtained is similar to that used by BRILES, MCGIBBON and IRWIN (1950), the latter reference providing a detailed account of the analysis of two antisera. The details of absorptions carried out by the author will be furnished on request.

The reagents obtained were assumed to contain a single antibody specificity. Progeny tests indicated whether the antigenic substance was inherited monofactorially and therefore indirectly the unity of the antigenic substance and the serological purity of the reagent. Only progeny from dihybrid or higher order testcross matings were used since such progeny furnished a maximum amount of information with a minimum expenditure of reagent. The heterozygosity of a parent was assumed if the cells of some of its progeny were non-reactive with the reagents employed. Each reagent was tested with at least 20 progeny in two or more sire families, therefore, sampling errors involving small family size are believed to be at a minimum.

RESULTS

Specificity of reagents

Table 1 provides a summary of agglutination tests of progeny from testcrosses in which results with each reagent have been considered separately, i.e. a monohybrid testcross. The expectation from such matings is a 1:1 ratio of reactive to non-reactive progeny. The (±) class indicates doubtful cases which for the most part could not be retested. The probable causes for such weak reactions are numerous, including errors in technique, a functional disorder in the bird tested, etc. These

TABLE 1

Reactions of the cells of progeny in monohybrid testcrosses Hy:1:1

Reagents for	No. of sires	No. of dams	Phenotypic classes		
			+	0	±
M_2	3	4	63	48	
M_5	9	20	128	103	
M'_5	5	17	75	69	
O_2	10	37	133	127	
Q_4	7	25	120	114	2
Q'_4	7	23	21	26	
S_2	7	34	104	109	
S'_2	6	14	74	72	
S_3	11	30	90	106	
2	4	18	69	67	1
5	9	25	71	102*	7
7	8	24	51	57	2
8	4	10	38	38	1
9	13	35	87	93	

* $\chi^2_1 = 5.56$, 1 df., $P = .01$–$.02$.

doubtful reactions do not in any way alter the conclusion that all antigenic substances but #5 appear to be inherited in a simple mendelian fashion. Each of the reagents except anti-#5 presumably detected the antigenic product of a single locus. In that sense they can be regarded as unitary for the population sampled. Generalizations to other populations cannot be made until they are tested. Reagents which are designated by primes (M_5', etc.) are those which were found to be duplicates of reagents bearing the same letter and numeral.

In the antiserum analyses, each antiserum fraction was designated numerically, 1, 2 or 3 in sequence. Following the progeny tests the designations were altered to conform to the relations among the genes responsible for the antigenic substances; the latter designations were used throughout this paper. Antiserum fractions which could not be obtained in concentrations sufficiently high to be useful as reagents were not isolated, but still retained their initial designations.

Additional evidence for the serological purity of the reagents lay in the demonstration of their independence when comparing the results of agglutination of these reagents with the same array of bird cells. A portion of these results is presented in table 2 and a more complete table is available on request. These results confirm the identity of M_5 with M_5', Q_4 with Q_4' and S_2 with S_2'. Reagents anti-*S*3 and anti-#9 may possess a common specificity but anti-M_2, M_5, O_5, Q_4, S_2, #2, #5, #7 and #8 appear entirely distinct one from the other and from anti-S_2 or anti-#9.

Reagents for A_7, A_9, B_2 and B_6 were kindly provided by W. E. BRILES of Texas Agricultural and Mechanical College. Agglutination tests with anti-Q_4 paralleled

TABLE 2

Typing tests of the flock of the Department of Poultry Husbandry

Reagents for	Part A: Agglutination with cells of birds listed															Part B: % of birds whose cells reacted with reagents				
	3204	3212	E295	Q791	Q832	Q928	S570	3191	A2282	C98	C2685	C4253	C4254	E137	All breeds	Barred Plymouth Rocks	New Hampshire Reds	White Plymouth Rocks	Rhode Island Reds
M_2	0	0	0	0	0	+	0	+	+	+	+	+	0	0	38.4(112)[1]	16.0(25)	70.9(55)	0.0(14)	0.0(18)
M_5	0	+	+	0	0	0	0	0	0	0	+	0	0	0	11.6(112)	0.0(25)	12.1(58)	0.0(15)	42.9(14)
M_5'	0	+	+	0	0	0	0	0	0	0	+	0	0	0	10.6(113)	0.0(25)	14.5(62)	0.0(13)	23.1(13)
O_5	+	+	−	0	+	0	+	+	0	0	+	+	+	+	61.6(112)	29.6(27)	66.6(54)	60.0(15)	100.0(16)
Q_4	+	0	0	+	+	0	0	0	0	0	0	+	+	+	57.1(98)	87.5(24)	51.0(47)	45.5(11)	37.5(16)
Q_4'	+	0	0	+	+	0	0	0	0	0	0	+	+	+	57.5(106)	87.5(24)	51.7(56)	45.5(11)	40.0(15)
S_2	+	+	−	+	+	0	+	0	0	0	+	0	0	+	57.9(107)	21.4(28)	59.2(54)	88.8(9)	100.0(16)
S_2'	−	+	0	+	+	0	+	0	0	0	+	0	0	+	58.4(101)	21.4(28)	61.2(49)	88.8(9)	100.0(12)
S_3	0	+	0	0	0	0	0	+	0	0	+	+	0	+	8.7(104)	0.0(24)	12.3(49)	0.0(14)	17.6(17)
2	0	+	+	0	0	±	+	+	0	+	0	0	0	+	44.4(108)	4.3(23)	35.0(57)	92.3(13)	100.0(15)
5	+	+	0	+	+	0	+	+	0	0	+	+	+	+	78.4(102)	87.5(24)	72.0(50)	60.0(10)	94.4(18)
7	−	−	−	−	−	−	−	+	+	+	0	0	0	0	−	−	6.5(31)	−	−
8	0	+	+	0	0	+	0	0	0	0	0	+	+	0	−	−	62.5(8)	−	−
9	−	−	−	−	−	−	−	+	0	0	0	0	0	+	28.2(79)	4.5(22)	35.5(45)	25.0(4)	25.0(8)
A_7	0	+	0	0	0	+	+	−	−	−	−	−	−	−	−	−	−	−	−
A_9	+	+	0	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B_2	+	+	0	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B_6	+	+	0	+	+	−	0	−	−	−	−	−	−	−	−	−	−	−	−

All agglutinations indicated by (+) regardless of degree of reaction. No reaction is designated by (0). Where test was not performed a dash is used.

[1] Number tested in parentheses.

those with anti-B_6 (table 2) with four exceptions in which the cells reacted with anti-B_6 but not with anti-Q_4; one of the latter cases is shown in table 2. These reagents cannot, therefore, be considered identical but may possess an antibody specificity in common. Additional tests will be necessary to determine the relationships between anti-A_7, anti-A_9, anti-B_2 and those prepared by the author. The results of typing tests with the parents used in the progeny tests are summarized in part B of table 2. The latter section shows the number tested and the frequency of reactors of the various breeds used. The cells of all of these birds were used in comparisons typified in part A of table 2.

TESTS FOR GENE INDEPENDENCE

The antigenic substances appeared to be inherited in a simple monofactorial fashion. It was, therefore, of interest to determine whether the causative genes were independent or whether any of them were linked or allelic to one another. The data presented in table 3 were obtained from dihybrid testcrosses in which the expectation for the progeny was 1:1:1:1 with an hypothesis of independence. The recombination frequency observed between M_2 and Q_4 was 5.4 ± 4.2% and between O_5 and S_2, 2.6 ± 2%, indicative of close linkage. S_2 and S_3 segregated as alleles and O_5 and S_3 segregated as if completely linked in the coupling phase. In an analogous fashion M_2 and M_5 segregated as if they were alleles. Independent segregation was noted in matings with M_3 or M_5 on the one hand and either O_5, S_2 or S_3, members of the second linkage group on the other hand. There appear to be a minimum of four

TABLE 3

Reactions of progeny in dihybrid testcrosses Hy:1:1:1:1

Reagents for	No. of sires	No. of dams	Phenotypic classes				
			++	+0	0+	00	x^2P
$M_2\,M_5$	1	3	0	14	15	0	<.01
$M_2\,O_5$	2	3	3	3	6	7	.3–.5
$M_2\,Q_4$	3	14	4	59	46	2	<.01
$M_2\,S_2$	2	7	8	12	8	11	.7–.8
$M_2\,S_3$	3	9	24	26	18	23	.5–.7
$M_5\,O_5$	2	2	10	12	6	6	.3–.5
$M_5\,S_2$	3	6	24	27	18	18	.3–.5
$M_5\,S_3$	2	4	13	12	13	13	>.99
$O_5\,S_2$	8	34	113	3	3	109	<.01
$O_5\,S_3$	3	4	33	0	0	55	<.01
$O_5\,2$	1	2	4	6	4	3	.7–.8
$O_5\,7$	3	14	22	31	31	23	.3–.5
$Q_4\,S_3$	1	1	14	6	21	19	.02–.05
$S_2\,S_3$	1	5	0	41	39	0	<.01
$S_3\,2$	2	6	15	15	10	12	.5–.7
$S_3\,5$	2	3	5	8	6	5	.8–.9

++—Cells possessing both substances
+0—Cells possessing the first but not the second
0+—Cells possessing the second but not the first
00—Cells lacking both substances

loci with M_2 and Q_4 forming one linkage group and a second group comprised of O_5 and S_2. Further studies are necessary in order to elucidate the chromosomal relations of the genes for substances ✻2, ✻5, ✻7, ✻8 and ✻9.

In all progeny tests made thus far, genes O_5 and S_2 have been closely associated in apparently the coupling phase of linkage with the exception of the observed crossover types. This close association is not evident from more recent progeny tests. One sire, ✻3212, is apparently homozygous for the O_5 locus since thirteen of his progeny possess the O_5 substance. However, there is segregation of the S_2 and S_3 substances among his progeny. Among 33 progeny in another testcross 14 possessed S_3 and 19 did not. In yet a third testcross with another group of hens, ✻3212 sired nine progeny five of which possessed S_2 and four did not. Whereas ✻3212 possessed O_5, S_2 and S_3 the hens to which he was mated lacked one or two but not three of these substances so that a simultaneous trihybrid testcross was not possible. The grandson, ✻A8406 ($O_5^+S_2^+S_3^+$) of ✻3212, sired progeny with a similar segregation pattern. In a testcross, 61 progeny were obtained all of which possessed O_5 but segregated with respect to the S_2 and S_3 substances as follows: 28 with S_2 and 31 with S_3. Thus there is further evidence that the O_5 and S_2 substances are genetically distinct.

In table 3 it was indicated that the linkage between the O_5 and S_2 loci was in the coupling phase and that between M_2 and Q_4 in the repulsion phase. Recently progeny tests were made of the linkage in a phase reciprocal to that indicated in table 3. A number of progeny were obtained from matings of a crossover containing O_5 but lacking S_2 with an unrelated female containing only S_2. From this mating two males were obtained which contained both O_5 and S_2 and they in turn were mated to females lacking both substances. This constituted a testcross in which the linkage of the O_5 and S_2 genes was in the repulsion phase. The results obtained to date are shown in part A, of table 4. As expected with this small sample the progeny fall in the two noncrossover classes.

In the M_2Q_4 system two hens of the crossover type containing both M_2 and Q_4 were available for mating but only one laid fertile eggs. The results of mating the latter individual (✻R999) to two males lacking M_2 and Q_4 are shown in part B, of table 4. It was expected that most of the progeny would segregate into two classes. Instead an apparent independent segregation was obtained.

Considering the closeness of linkage observed in the repulsion phase these results are surprising. It is possible that R999 contains a reciprocal translocation which would explain the observed findings. In such an event the M_2 gene would be found on a chromosome non-homologous to Q_4 and an apparently independent segregation would be obtained. Of 98 eggs obtained from R999 only 24 hatched of which only the 14 shown in table 4 were available for bleeding, the balance having died within a week after hatching, a datum which is of interest with respect to the possibility of translocation.

SEROLOGICAL PURITY, CROSSREACTION AND POSITION EFFECT

In addition to the tests of serological purity already considered the reagents which detected the crossover individuals were subjected to further analyses to determine

TABLE 4

Testcrosses of repulsion phase heterozygotes containing O_5 *and* S_2 *and coupling phase heterozygotes containing* M_2 *and* Q_4

A. Sires with O_5 and S_2	Dams without O_5 and S_2	Phenotypic classes			
		++	+0	+0	00
A9618	M580	0	3	3	0
A9618	R674	0	0	1	0
A9618	H1000	0	4	5	0
A9754	M578	0	1	1	0
A9754	R669	0	2	1	0
Total		0	10	11	0
B. Sires without M_2 and Q_4	**Dams with M_2 and Q_4**				
E1814	R999	2	2	3	4
3212	R999	0	1	2	0
Total		2	3	5	4

whether the postulated crossover progeny were merely serological artifacts, reacting with the reagent because of an antibody specificity not previously detected. The anti-M_2 reagent, for example, could be represented as containing anti-M_2 and an unknown type of antibody. If the reagents contained primarily one antibody specificity it should be possible to absorb the reagents completely with cells of birds possessing only M_2, or in the case of anti-Q_4 with cells of birds possessing only Q_4. Similar absorptions were carried out with reagents anti-O_5 and anti-S_2. The results of these absorptions are summarized in table 5; the detailed tables are available on request. Following absorption of the anti-M_2 reagent with the cells of an $M_2^+Q_4^0$ bird (a bird which contained M_2 but lacked Q_4) the reagent did not react with the cells of M_2Q_4 birds. Comparable results were obtained from absorptions with anti-Q_4, anti-O_5 and anti-S_2. Following absorption with the cells of a bird lacking both M_2 and Q_4 or O_5 and S_2 the respective reagents were still strongly reactive with the cells of birds possessing these substances. It may be concluded that the reagents anti-M_2, Q_4, O_5 and S_2 did not contain additional specificities which distinguished the crossover and non-crossover classes.

Since the genes M_2 and Q_4, and O_5 and S_2 respectively appeared to be closely linked it was of interest to determine whether crossreactivity existed between the antigenic products of the linked genes. Two criteria, cross absorption and reciprocal immunization, were employed to test for cross reactivity. With the criterion of absorption, the antigenic products of the linked genes would be considered crossreactive if each could absorb antibodies for the other antigen.

The cells of birds with the phenotype $M_2^0Q_4^+$ were used to absorb the non-homologous anti-M_2 reagent and reciprocally $M_2^+Q_4^0$ cells were used to absorb the anti-Q_4 reagent also non-homologous. Following absorption, the sera were titrated and the

TABLE 5

Summary of absorption results

Reagents for	Absorbed with cells of	Tested with cells of							
		$M_2^+Q_4^+$ (3)*	$M_2^+Q_4^0$ (13)	$M_2^0Q_4^+$ (6)	$M_2^0Q_4^0$ (1)	$M_2^+Q_4^+$ (3)	$M_2^+Q_4^0$ (13)	$M_2^0Q_4^+$ (11)	$M_2^0Q_4^0$ (3)
Part A anti-M_2 and anti-Q_4 reagents									
M_2	$M_2^+ Q_4^0$ (2)	0	0	0	0				
	$M_2^0 Q_4^+$ (4)	+	+	0	0				
	$M_2^0 Q_4^0$ (1)	+	+	0	0				
Q_4	$M_2^+ Q_4^0$ (4)					+	0	+	0
	$M_2^0 Q_4^+$ (4)					0	0	0	0
	$M_2^0 Q_4^0$ (4)					+	0	+	0

Part B anti-Q_5 and S_2 reagents

Reagents for	Absorbed with cells of	Tested with cells of							
		$O_5^+S_2^+$ (17)	$O_5^+S_2^0$ (9)	$O_5^0S_2^0$ (3)	$O_5^0S_2^+$ (2)	$O_5^+S_2^+$ (14)	$O_5^+S_2^0$ (2)	$O_5^0S_2^+$ (2)	$O_5^0S_2^0$ (1)
O_5	$O_5^+ S_2^0$ (3)	0	0	0	0				
	$O_5^0 S_2^+$ (3)	+	+	0	0				
	$O_5^0 S_2^0$ (2)	+	+	0	0				
	$O_5^+ S_2^0$ (2)					+	0	+	0
S_2	$O_5^0 S_2^+$ (1)					0	0	0	0
	$O_5^0 S_2^0$ (1)					+	0	+	0

* Number of birds tested and the number of absorbed reagents used are shown in parentheses.

tested cells reacted with these sera to a degree comparable to reagents absorbed with the cells of birds lacking both M_2 and Q_4 substances. These results are summarized in table 5 and the details will be furnished on request. Absorptions of this kind were also carried out with reagents anti-O_5 and S_2. The results obtained paralleled those from absorptions of anti-M_2 and Q_4 in that cross reactivity or antigenic similarity between O_5 and S_2 was not detected.

A further test of the specificity of the substances produced by the linked genes was afforded by reciprocal immunization between birds possessing only one of the two substances, e.g. $O_5^+S_2^0 \leftrightarrows O_5^0S_2^+$ and $M_2^+Q_4^0 \rightleftarrows M_2^0Q_4^+$. All birds which were immunized produced antisera which contained a number of different antibody specificities. In order to obtain a reagent specific for each of these antigenic substances differential absorptions were necessary. Duplicate reagents for each of the substances, M_2, Q_4, O_5 and S_2 agglutination tests gave results which paralleled those with anti-O_5, anti-O_6, anti-M_2 or anti-Q_4. Since specific sera were obtained following immunization this provided evidence against the similarity or crossreactivity of antigens associated with the linked loci.

Subsequent to the matings considered in table 3 birds were obtained which were heterozygous for O_5 and S_2. In some of these birds the genes were linked in the coupling phase and in others in the repulsion phase, thus permitting a test for the presence

of the cis-trans form of position effect. If contrasting but unknown alleles are depicted with the subscript "x", the cis form could be represented as $\frac{O_5S_2}{O_xS_x}$ and the trans form as $\frac{O_5S_x}{O_xS_2}$. The criterion for position effect was the amount of O_5 and S_2 substance possessed by each genotypic class. The difference in amounts were estimated by titration and absorption of reagents against O_5 and S_2 with the cells of the respective heterozygotes. No significant differences could be detected in titrations with anti-S_2 and anti-O_5S_2, the latter reagent containing both specificities. A typical titration with one of the 11 repulsion phase heterozygotes with reagent anti-O_5S_2 is as follows: $\frac{1}{3}$-3; $\frac{1}{6}$-3; $\frac{1}{12}$-3; $\frac{1}{24}$-2 (the fraction shows the proportion of reagent in the total volume of reagent and diluent with the integer the agglutination score at the indicated dilution). Of the three coupling phase heterozygotes, a typical reaction is the following: $\frac{1}{3}$-4, $\frac{1}{16}$-3, $\frac{1}{12}$-3, $\frac{1}{24}$-3. With the reagent for S_2 the repulsion phase heterozygote, $\frac{1}{2}$-2, $\frac{1}{4}$-2, $\frac{1}{8}$-1, $\frac{1}{16}$-0 and the coupling phase heterozygote: $\frac{1}{2}$-3, $\frac{1}{4}$-2, $\frac{1}{8}$-+, $\frac{1}{16}$-+. Nor were significant differences detected in agglutination when absorptions of anti-O_5S_2 were carried out with 4 repulsion phase and 3 coupling phase heterozygotes. The details of these tests will be furnished on request. These tests indicate that detectable differences were not present in the amounts of either or both antigens (O_5 and S_2) possessed by the two classes of heterozygotes although variations in the amounts of antigen may be present within each group. In order to detect altered antigenicity or change in cross reaction of the O_5 and S_2 antigenic specificities it would be necessary to obtain birds which differ genetically only with respect to the positions of the O_5 and S_2 genes; such stocks are not now available.

DISCUSSION

Studies of linkage in man involving genes for cellular antigens have been reviewed by RACE and SANGER (1954). Among the 42 studies was the proposal of linkage between the Lutheran and Lewis loci first reported by MOHR in 1951. The data reported by MOHR included one family containing a crossover, the suspected crossing over having occurred in the father. RACE, LAWLER and PENROSE, quoted in MOHR (1954) also tested a family in which a crossover type was observed.

A number of families have been studied for the relation of the *Rh* locus with ovalocytosis, with indications that the two loci are linked (LAWLER 1954; CHALMERS and LAWLER 1953; GOODALL, *et al.* 1953; and MARSHALL, *et al.* 1954). One family was reported (LAWLER 1954) in which the segregation indicated independence of the two loci. As an explanation for this exceptional family it was suggested either that two loci were responsible for the ovalocytosis condition or some chromosomal aberration such as translocation had taken place. In a study involving four Japanese families, FUJII, MOLONEY and MORTON (1955) did not find significant evidence of linkage between ovalocytosis and the *Rh* system. Since the sample was quite small the authors make a plea for continued study of the relation between *Rh* and ovalocytosis. RENWICK and LAWLER (1955) reported on a study of families in which the nail-patella syndrome occurred. They found significant linkage between the locus for this syndrome and the *ABO* locus. As additional markers are discovered and newer statistical techniques are employed such as sequential analyses (WALD 1947; MORTON

1955) further studies will be conducted which may lead to new discoveries of linkage in man.

Attempts to find additional linkages in chickens involve the difficulty that the chicken possesses a large number of chromosomes, on the order of 39 pairs. There is the possibility, however, that in this species the genes are present exclusively in the 7 large chromosomes, the dot-like chromosomes are dispensable (NEWCOMER 1953), a condition favorable to the discovery of linkage. There are two approaches which might be more productive in the search for antigenic products of linked loci. The first of these is to look for reagents which among testcross progeny detect an excess of offspring whose cells react with the reagent. The excess of reactors would be less than the three to one ratio expected in the case of independent genes and slightly more than the one to one ration expected for a single gene. For small samples of the order of 20 to 30 the deviations from 1:1 would probably not be significant statistically.

All linkage studies of blood group genes with genes for other characteristics followed the discovery of the cellular antigen. Immunological procedures permit the acquisition of linkage information without the necessity of first finding the cellular antigen. This would have great value in human genetics where it is desired to determine an antigenic marker linked to a gene for a physiological trait. An example with chickens will illustrate this method. The blood of a bird known to be heterozygous for rose comb for example would be injected into a single comb bird. The immune sera obtained from the latter chicken would be absorbed with pooled cells of all single comb progeny from the mating of rose comb with single comb. This absorption is made to eliminate all antibody specificities except those for antigens whose genes are closely linked to the rose comb locus. Remaining specificities should be reactive only with the antigenic product of the linked gene. In a similar way all of the chromosomes in the fowl could be examined for the presence of blood group genes as they are linked to genes marking the chromosomes.

This type of experiment is similar in principle to the one carried out by LEVIT, *et al.* (1936) in which serological differences were reported between XX and XXY females in Drosophila. An experiment was carried out by the author to determine if blood group genes are located in the "X" chromosome and the postulated "Y" chromosome of chickens. The assumptions involved were (1) that blood group genes are randomly distributed and that some would be found in the X chromosome and the Y chromosome, if the latter is present in the chicken, and (2) that blood group loci in the X chromosome would contain as many different alleles as has been found for other loci studied (BRILES, *et al.* 1950). With the latter assumption in mind, breed crosses were made. The matings and the number of progeny available for testing follow: Buff Orpington × Single Comb White Leghorn (39), S. C. Barred Plymouth Rock × S.C. White Leghorn (68), Silver Oklabar × S.C. White Leghorn (31), New Hampshire Red × White Plymouth Rock (57). Such matings provided a wide array of genotypes for sampling and permitted the detection of locus effects if more than one allele were present. The mating of a New Hampshire Red male to a White Plymouth Rock female involved only the two individuals but in all other matings a single male was mated to five to seven S.C. White Leghorn females all from the same

or related inbred line. The immunizations were made by injecting the cells of a female into the sire and obtaining an immune serum. Absorptions were carried out with the pooled cells of the progeny of each sex as well as by a series of absorptions with the cells of individuals from each sex. In all cases when absorption removed antibodies for all the members of one sex the absorbed sera did not react with the cells of members from the opposite sex. It may be concluded that if blood group genes are located in either the X or Y chromosomes of chickens their products are so weakly antigenic that detectable antibodies were not produced.

A program of gene mapping would be highly desirable for the solution of many problems confronting immunogeneticists. Correlations between blood groups and physiological traits have been reported by BRILES *et al.* (1953) and SHULTZ and BRILES (1953). These correlations might be due to linkage of genes responsible for the characters, but even if they were not, linkage information would be of value in attempting to understand the nature of the correlation.

As close linkages are found the possibilities become greater for testing for the presence of cross reaction, which it is hoped will lead to the solution of a fundamental immunogenetic question, "What is the nature of antigenic specificity imparted by the gene?"

SUMMARY

Two independent linkage groups involving blood group genes have been found in the chicken. A recombination frequency of 5.4 ± 4.2% has been found between M_2 and Q_4, and of 2.6 ± 2.0% between O_5 and S_2. S_3 has been found allelic to S_2 and M_5 allelic to M_2. Reagents for four other antigenic substances have been obtained but the linkage relations of the responsible genes have not been determined.

ACKNOWLEDGMENT

The author is grateful to DR. M. R. IRWIN for his many helpful criticisms and suggestions in the preparation of this paper and to DR. W. H. MCGIBBON for the use of poultry stock and equipment.

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LINEAR INHERITANCE IN TRANSDUCTIONAL CLONES[1]

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THE rule of inheritance in cell lineages is the transmission of an undiminished legacy to each of a geometrically increasing family of descendants. Episodes of mutation or segregation may intervene, but further descendants will again follow this rule of clonal heredity, which is the corollary of equal division. But other rules of inheritance are known—for example, the entailment of estates in land and the traditional law of primogeniture in titles of nobility—whereby a legacy must pass undivided through a single *line* of descent through the generations. This paper will have to do with biological analogies of linear inheritance which have appeared in experiments on the transduction of motility-genes in Salmonella.

Transduction is a mechanism of genetic recombination which is notable for the transfer of hereditary fragments from one cell to another (SYMPOSIUM 1955, LEDERBERG 1956a). In these experiments, a temperate bacteriophage serves as vector for the fragments, which are furnished by the disruption of the chromosomes of a bacterial host as it supports the growth of the phage. When this crop of phage is applied to cells of a suitably marked recipient strain, some ($\sim 10^{-6}$) of these cells yield a transformed clone which carries a given marker from the donor. In previous studies, the transformed clones have exhibited the same genotypic stability as did the parents. However, the selective methods which were used to isolate the rare recombinants would overlook transductional effects that did not yield substantial clones of the new types. These studies included auxotrophic, fermentative, resistance, serological and motility markers, and each one for which a suitable selective technique was available was subject to transduction in much the same fashion.

The following experiments are a follow-up of observations on "motility trails" (see paragraph 1. 1) initiated by DR. BRUCE STOCKER during a research visit to this laboratory (STOCKER, ZINDER and LEDERBERG 1953). After his return to England, DR. STOCKER began microscopic studies on these trails; the immediate concern here was the problem of segregation and crossing over in transductional clones. However, the two studies proved to be operationally inseparable. I am indebted to DR. STOCKER for an unreserved exchange of materials, information and manuscript drafts throughout these studies. In the main, the terminology also follows his suggestions. A concordance of my results and interpretations with his (STOCKER 1956b) is given at the close of this paper.

Glossary and symbols. The central concept of this paper is that of a *line* (adj. *linear* or *unilinear*) which signifies a single, unbranched, finite or infinite chain of descent

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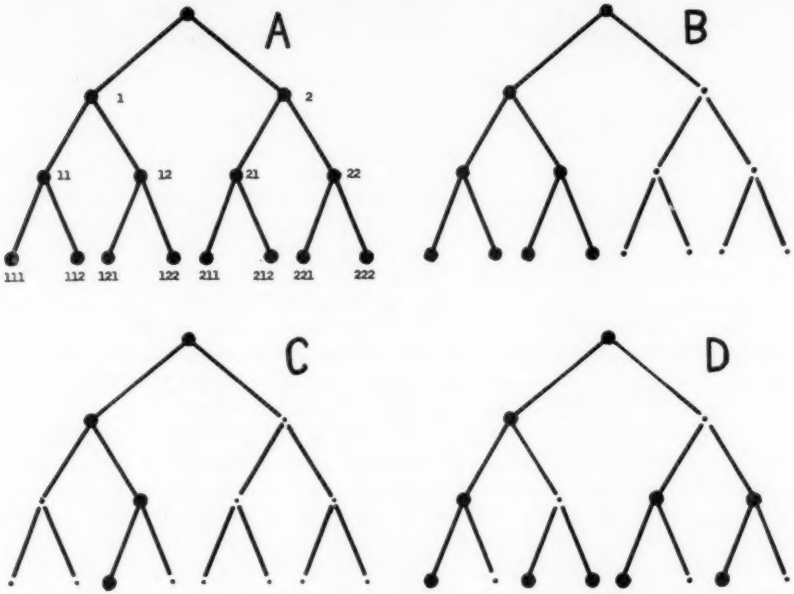


FIGURE 1.—Diagrammatic representations of hereditary transmission within a clone[2]. A, *holo*-clone. B, segregating clone. C, line. D, a pattern of delayed development, see 4.5. The large circles may be taken as +, the small as −.

(fig. 1C), with regard to a given quality. A line must correspond to a sequence of unequal fissions, at each stage of which only *one* product propagates the line. A clone may be *multilinear* if its descent can be resolved into a number of lines. By extension, a clone or a cell may be loosely referred to as line or linear if it contains or initiates a line. In previous discussions, *semiclone* and *chain* have been used as synonyms of *line*. As will be seen, trails are the overt manifestations of linear inheritance of motility in a clone growing in semisolid agar medium.

Clone is taken to mean the progeny from a single cell, and often implies the regular appearance of a trait throughout that progeny. When the meaning is not given by the context, *holoclone* (see fig. 1A) will be used for the latter sense.

Exogenote signifies a chromosome fragment, explicitly the one given over in transduction. *A* — x *B*, or its equivalent, *B* x—*A*, are abbreviations for transduction from genotype *A* (donor) to *B* (recipient). These and other terms are discussed in more detail elsewhere (MORSE, LEDERBERG and LEDERBERG 1956b).

[2] A uniform convention for the numbering of cells in a lineage would be helpful. The scheme shown in A is adapted from JENNINGS (1908; cf. ZELLE 1951) and has the advantage that the number of digits is the generation number, and that the family relationships are readily visualized. For very large pedigrees a sequence of *binary* numbers could be replaced by the corresponding *decimal*, but the generation number must then be specified. In C, the line shown is 0-1-12-121. In principle, however, any single line could be written 0-1-11-111-. . . as has been adopted in table 2.

Following the introduction, this paper comprises the following: Section 1 (¶¶1.1 to 1.3) reviews the experimental procedures. Section 2 gives experimental detail on the microscopic analysis of cell pedigrees, and section 3 on plating experiments. Section 4 brings together ancillary observations. The interpretive analysis of the data and speculations are deferred to section 5. Section 6 compares DR. STOCKER's data with these. Many readers may wish to proceed to the recapitulations of the experimental sections, placed for convenience at 2.0 and 3.0, and to the discussions of sections 5 and 6, before reviewing the details.

1. MATERIALS, METHODS AND PRELIMINARY OBSERVATIONS

1.1 General procedures and background observations have been given at length (ZINDER and LEDERBERG 1952; STOCKER *et al.* 1953; LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956) and will be recapitulated only briefly. In 1897, HISS had discovered that a very soft agar permitted motile bacteria to spread throughout the medium, while nonmotile varieties stayed where put. The technique was rediscovered several times, and has since been widely adopted in enteric bacteriology. The spreading cloudy growth of a motile clone is called a swarm (figs. 7, 8) and is the most characteristic result of transduction of motility to a nonmotile strain (STOCKER *et al.* 1953). In the same experiments, trails are seen: these are groups of small colonies strung out through the soft agar for some millimeters from the point where the treated bacteria of the nonmotile strain had been planted (fig. 4). The trails were thought to mark the path of a motile cell wherever it divided and left behind nonmotile progeny. The first observations suggested that the trails, and therefore the corresponding lines of inheritance of motility, were always unbranched, but later evidence has weakened this conclusion. At the time, however, the trails were explained by the linear transmission through the recipient clone of a damaged exogenote which could no longer replicate, but could still confer motility on the cell which carried it. Since transduction here fell short of a transformed clone, it was described as abortive. To test the hypothesis of abortive transduction and to supplement plating experiments, cell lineages from transformed motile cells were studied more directly in pedigrees controlled by micromanipulation.

1.2 The manipulative procedures, especially the use of the oil chamber, follow DE FONBRUNE (1949). The principal media were Difco penassay broth and NGA, "nutrient gelatin agar", 0.8 per cent gelatin, 0.4 per cent agar with a broth base. Microclones were routinely held at room temperature (22 ± 2°C) and examined at 150 magnifications darkfield. This was conveniently obtained with a 15 × ocular and a 10 × objective (not necessarily phase contrast) in combination with a Bausch and Lomb LWD phase condenser carrying a 43 × annulus. For closer study at 645 ×, a matched 43 × dark phase contrast objective was swung into place.

1.3 Most of the experiments were of the form SW-623 —x SW-666. The cultures are described more fully elsewhere (LEDERBERG and EDWARDS 1953) but both are derived from a monophasic *S. paratyphi B*. SW-666 is Fla_1^- H_1^b (flagellaless, hence nonmotile; phase-1 flagellar determinant b); SW-623 is Fla_1^+ H_1^i. The transducing phage was PLT22 adapted by serial passage on SW-623. SW-666 was originally chosen for these experiments because of the previously demonstrated linkage of the

H_1 to the Fla_1 factor, a consideration which is immaterial except where it is emphasized below.

EXPERIMENTAL RESULTS AND CONCLUSIONS

2. Experiments with microclones

2.0 *Recapitulation.* Partial pedigree analyses were made of the progeny from isolated motile initials. A single initial might generate many motile offspring during the first ten or fifteen generations. When these motile offspring were isolated and propagated, they usually constituted strict hereditary lines (for motility) for as many as thirty fissions longer. In further pedigree analyses, the number of lines which issued from two sister cells was unequally partitioned. That is, if "*E*" represents the potentiality to produce *many* motile offspring, *E* was unequally, perhaps linearly, transmitted at cell division. Formazan granules were also transmitted linearly, but the possible inherent polarity of the bacteria, as marked by formazan, could not be correlated with the linear transmission of *E*. About four per cent of motile initials generated stable transductional clones. These often showed an early segregation of motile and nonmotile subclones. The concurrence of motile and multilinear subclones was also noted.

2.1 *Isolation of motile initials.* The first step in each experiment was the isolation of infrequent motile *initial* cells from SW-623 —x SW-666. Equal volumes of an overnight broth culture of SW-666 and stock lysate of SW-623 were mixed and incubated for about 90 minutes to give an input of about 10^9 bacteria, 10^{10} phage per ml. Between 90 and 150 minutes, 10^4 to 10^5 cells became motile. These initials were readily isolated with the help of a trapping droplet. Small drops of the treated cultures were deposited on a cover glass under oil, adjacent to drops of clear broth. The droplets were then fused in pairs, permitting the motile initials to swim into the traps where they could be clearly seen and individually caught. The interval between mixture and isolation was two to three hours, which might allow as many as three fissions depending on a variable initial lag. Subsequent pedigrees are therefore likely to be truncated at the origin. After most of the experiments were completed, it was found that isolation was simplified by spinning down the transductional mixture after about 60 minutes and trapping from droplets of concentrated sediments. Hundreds of initials could be trapped in a short time by this procedure, which was used in experiments below 2.16, 2.18, 3.1, 3.7, 4.1.

2.2 *Undivided clones.* The simplest experiment was to plant the motile initials in individual droplets and examine the microclones the next day, when they usually contained about 10^4 cells each. Table 1 shows the results from 384 viable isolations, including some pedigrees which have been summed to give the total yields. In addition, about ten per cent of the cells isolated were inviable. In these cases, a long filament ("snake") often persisted which might remain motile for hours or days but never divide. Other clones contained one or several ghosts which may have originated by phage lysis.

2.3 Only fifteen (four per cent) of the clones contained a preponderance of motile cells which would relate them to swarms, i.e., holoclonal inheritance of motility These will be taken up later 2.22.

TABLE 1

Distribution of yields of motile cells in 384 single microclones

Yield:	0	1	2	3	4	5	6	7	8	9	10	11–15	16–20	21–30	>30	Swarm
Number of clones:	100	59	39	28	17	7	9	6	6	4	6	25	23	19	21	15

2.4 The remaining clones all had a limited number of motile individuals, ranging with decreasing frequency from 0 to about 100. The distribution of these numbers was highly skewed, as can be seen from table 1. For numbers in the range 0 to 9, the distribution is approximately exponential, each class being proportional to 0.8^x; while the larger numbers follow a nearly flat distribution. Except as an indication of nonhomogeneity, the quantitative significance of this distribution is not apparent, and we shall be concerned only with its qualitative features.

2.5 The microclones therefore give a partial corroboration of the hypothesis of abortive transduction of motility, in so far as transformed motile cells are isolated which do not transmit the trait regularly to their descendants. The frequent class of clones, containing one motile individual is most readily understood: each comprises a line still in being after 13 fissions ($2^{13} \sim 10^4$), while the most frequent, zero class consists of lines that had terminated some time between the first and the 13th fission. Most of the experiments are directed at an understanding of the remaining two thirds of clones which have more than one motile individual: whether these are multilinear, and if so the patterns of distribution or generation of the lines.

2.6 *Progeny of intermediate isolates.* These experiments were done to confirm the linear inheritance of motility for a number of generations, and to ascertain whether the clones containing many motile cells could be resolved into lines, or whether they would show a continuing pattern of irregular transmission which might be neither linear nor clonal. The expression "isolation at n_k" means that a motile individual was reisolated from a clone whose total population indicated a history of k fissions. As indicated in the previous paragraph, many isolates were made at about n_{10} to n_{13}; the history of these pedigrees is summarized in figure 2.

2.7 As shown in figure 2, linear inheritance was followed strictly in every reisolate but one after n_{11}, that is, no branching (production of two motile progeny from one cell) *need* have occurred after n_{11} or *could* have occurred after n_{15}. This range, n_{11} to n_{15}, follows because the clones could not be examined at each fission. Many of the clones were already resolved into one or more lines between the first and tenth fissions. The exceptional clone (*A* in figure 2) must have had a branch not earlier than n_{19} and possibly as late as n_{27}; the figure represents a progeny from a cell isolated at n_{15} which gave a subclone containing 18 motile among 5,000 nonmotile cells. As $18 \sim 2^4$, and $5000 \sim 2^{12}$, this subclone must have branched not earlier than $n = 15 + 4 = 19$, and not later than $n = 15 + 12 = 27$. Ten of the motile cells at n_{27} were isolated; one formed a line for at least 13 additional generations (n_{40}); the others were nonmotile or inviable at the next examination at n_{30} to n_{41}.

2.8 The outstanding examples of continued linear inheritance were a pair of motile cells isolated from the same clone at n_7 which gave regular asymmetric division for

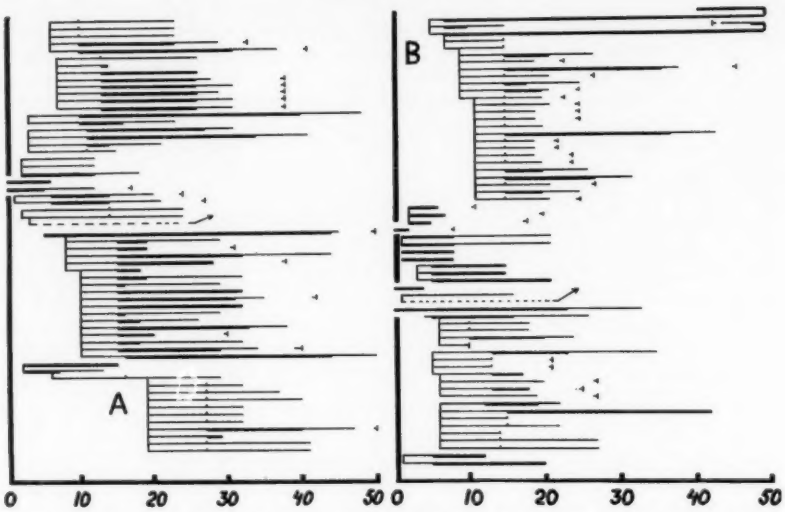


FIGURE 2.—Duration of motile lines generated by isolated initials. The scale is the number of generations elapsed from the time of isolation. The heavy horizontal bars show the minimum duration of a single line, from the last point at which any branching could have occurred to the earliest point at which the line could have terminated. The light bars and extensions show (to the left) the earliest point at which branching could have ceased, and (to the right) the longest that each line might have lasted. The overlap represents intervals during which individual clones were not examined. A vertical tick on a light bar is equivalent to a short heavy bar, that is, a single motile cell was observed and isolated at this point, but had no motile progeny when its subclone was re-examined. Many lines were terminated before motility had ceased; when examinations were continued after the cessation of motility in a line, the end of the study is indicated by +. An arrow indicates a swarm. Vertical bars connect lines that had been isolated from the same clone; they do not represent the derivation of one line from another.

an additional 45 and 52 fissions, respectively (fig. 2B). These lines were observed and reisolated at intervals of a few fissions until they finally terminated, in one case by cessation of growth, in the other by the gradual loss of motility. (This experiment lasted seven days (March 17–23, 1954), the clones being held at 10°C to slow their growth at night.)

2.9 These pedigrees, from intermediate isolates, suggest that clones with many motile cells are indeed multilinear, i.e., can be resolved into simple lines; although branching may persist as late as the third decade of fissions, it is usually not observed after the first. Once established, the lines were propagated for a variable interval (from one to 42 fissions) being terminated either by the death or immotility of the line cell.

2.10 On a number of occasions, a motile line was watched throughout one or more fission intervals. The dividing cell remained active until the moment of separation, at which point one daughter continued to move, while the other remained stationary. The motile daughter was the cell which continued the line on further division.

2.11 *Early lineages from motile initials and partition of line numbers at fission.* It is manifestly impossible to make a complete pedigree analysis of a multilinear clone for more than a few generations, if for no other reason than that one cover glass will not hold more than a few dozen isolates, and that at least a minute is needed, under the best circumstances, to separate two daughters into separate drops. As the clones grew, it became increasingly difficult to complete the handling of a generation before it divided again, and to maintain a coherent record of its disposition. On the other hand, concentration of effort on a single clone was unrewarding because the majority of initials generate none, one or very few lines. No procedure was found (cf. STOCKER, and 4.9) by which the minority of cells that would generate multilinear or swarm-clones could be detected in advance of growing out their progeny. A number of clones were, however, followed for a few fissions to answer the specific question whether the partition of lines (i.e., the total number of motile cells which ultimately appear in sib subclones) was random at the division of a multilinear cell. Figure 3 represents these partitions most of which come from single observations at an early fission. The coordinates of each point give the larger yield as abscissa, the smaller as ordinate. Thus the point (40, 2) refers to a fission at which one daughter gave a clone (usually read at a size of 10^3 to 10^4) containing 40 motile individuals, the other daughter clone giving 2. In a few cases, the partition could be followed for two or three fissions. Only a few of the partitions represent divisions later than n_{10} for the reasons given in 2.7.

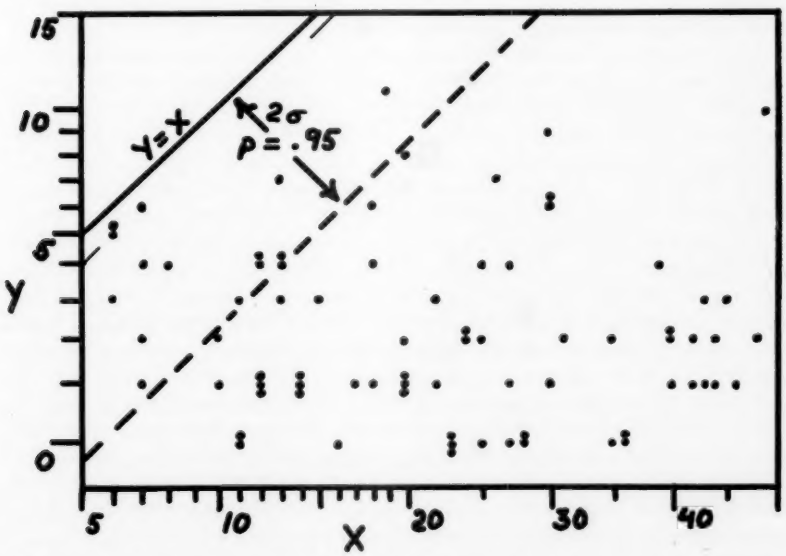


FIGURE 3.—Partition of potential lines at cell division. Each point represents a fission after which the two daughters were allowed to form clones, and the yield of motile lines in each was enumerated. The smaller yield is always given as ordinate. For details of the method of plotting, see 2.12.

2.12 The figure presents only those partitions where a total of eight or more lines was at stake. The data are plotted on a modification of MOSTELLER and TUKEY's (1949) co-ordinate paper. The ordinate scale is shifted to $\sqrt{y+1}$ rather than $\sqrt{y}$ so that the "shortest distances" can be read directly from the plotted points. The "2σ" band which lies parallel to the expected line, $x = y$, thus should exclude only five percent of the observations. The observed partitions are clearly unequal, one daughter tending to inherit most of the potential lines. The most nearly equal splits of a large stake were 19,11 and 30,9; grossly discrepant splits such as 36,0 or 40,1 were more common.

2.13 These inequalities are consistent with the hypothesis (STOCKER 1956b) that the multilinear clones are resolvable into two orders of linear inheritance, the simple motility lines already described, and in some clones, a line defined by the ability to generate *many* of these simple lines. Following STOCKER, descents with many motile cells will be designated as the "*E* line" (*E* for exceptional), leaving open the question whether the descendancy is strictly linear (see 5.3).

2.14 *Linear inheritance of formazan residue.* Linear inheritance is the expected consequence of the passive handing down of a particle at cell division. An almost trivial instance of a line stems from observations on the bacterial reduction of triphenyltetrazolium chloride. The reduction product is triphenylformazan, a fat-soluble, water-insoluble red pigment which is usually deposited as a single conspicuous granule near one pole of the bacterium (LEDERBERG 1948; WEIBULL 1953). The transmission of this granule at cell division has been seen to be linear in direct pedigree isolations, and by other procedures 4.7.

2.15 *Correlation of motility with formazan-lines.* The mere fact of unipolar deposition of formazan speaks for a polarity of cellular organization in Salmonella (and other enteric bacteria) which belies the superficial antero-posterior symmetry of the rod. An effort was made to correlate the unequal division of a cell into formazan-carrying Fz^+ and not-carrying Fz^- daughters, with the unequal division of motility and of *E* lines. However, it proved to be difficult to induce the deposition of formazan in cells of microclones: in general, it is taken up only by cells in the stationary phase, and it was therefore impractical to study the correlation of formazan with motility in intermediate isolates. Nevertheless, cells already Fz^+ were amenable to transduction, so that pre-stained initials could be isolated. It was however noted that recipient populations, in which half the cells were Fz^+, gave motile initials of which only one to two percent were Fz^+. A similar negative correlation was found between motility and Fz^+ in experiments with motile clones of Salmonella and of *Escherichia coli*. Such clones invariably contain a proportion of temporarily nonmotile individuals which is increased after formazan-staining. However, those motile cells which do carry a unipolar granule appear to be as vigorous and viable as the controls. Possibly the formazan is toxic when it is deposited above a threshold level. No preferred orientation (formazan anterior or posterior) was noted; an individual motile cell might reverse its orientation at intervals of a few seconds.

2.16 Motile initials were collected from transductions to prestained recipients and followed for three or four fissions along the formazan line. That is, at successive fissions, the Fz^+ cell was separated for further observation, and the Fz^- sib set aside

TABLE 2

Yield of motile progeny in pedigree lines of formazan-stained initials

Initial no.	Yield in cell no.			
	2	12	112	Fz = 111
1.	4	25	0	inviable
2.	15	1	2	0
3.	23	0		0
4.	18	0	2	2
5.	13	1	1	2
6.	2	3	44	1
7.	0	22	0	1
8.	swarm	—	0	22
9.	2	18		6
10.	1	2	42	(2)
11.	3	1		12
12.	1	1	0	0
13.	3	0	0	0
14.	1	0	1	0
15.	1	0	1	0
16.	3	0	0	0
17.	3	0	2	1
18.	0	1	1	inviable
19.	2	0	2	0
20.	2	0	0	0

From pre-stained initials, the line of cells (1-11-111) carrying a formazan granule was followed for three fissions, the successive sibs (2-12-112) being transferred to individual droplets. Lines No. 3, 9 and 11 grew more slowly or rapidly than the others, and were therefore not separated at a third fission. Lines 1 to 9 are summarized in 2.16. Line 11 is excluded because of the uncertainty whether 111 or 112 would have been multilinear. In line 10, the granule was no longer discerned after the first fission, and the "formazan-line" was continued only arbitrarily. 12. to 20. are some of the pedigrees with few motile progeny.

In line 8, the initial cell had already divided twice when the isolate was reexamined, and cells 2 (= 21 + 22) and 12 were pooled. Unfortunately, the swarm that resulted was lost before it could be analysed for homogeneity.

The numbering of cells follows figure 1A.

and allowed to form a microclone. Finally, both sibs were set aside, and all the clones were scored for number of motiles the following morning. The sequences of table 2 may be thought of as progressive halvings of the initial cell, from the center to the polar granule. With three fissions, a random disposition would lead to the occurrence of *E* in successive segments (i.e., 2, 12, 112, 111, see fig. 1A) in the proportions 4:2:1:1. The eight *E*-clones of this experiment were found in the proportions 4:3:1:0, which is concordant with the expectation, i.e., the *E* quality is not specifically associated with a part of the initial cell that is marked by Fz polarity. The possibility of mutual exclusion of *E* and the Fz-marked segment was considered, but another clone (which also contained a swarm 2.22) included a multilinear progeny from the Fz-terminal segment.

2.17 Clones with few lines from the same experiment are also tabulated but show no striking features.

2.18 *Manifestation of linked transduction; tests for reciprocal crossing over.* As already mentioned the *Fla*$^-$ locus is linked to another marker, H_1, the parental couplings in these experiments usually being *Fla*$^+H_1{}^i$—*x Fla*$^-H_1{}^b$. To this point we have considered only the motility phenotype (*Fla*$^+$ or *Fla*$^-$). The serotype ($H_1{}^i$ or $H_1{}^b$) of motile clones is readily detected with absorbed antiserums by agglutination tests, or by the inhibition of motility in NGA or under the microscope. The *Fla*—H_1 linkage is exhibited by a proportion of clonal *swarms* which were *Fla*$^+H_1{}^i$ as well as others which were *Fla*$^+H_1{}^b$. The serotype of non-clonal initials and *lines* is now in question. The first trials showed a complete inhibition of motile initials equally by *b* and *i* serum, and in NGA as well as in microclones. However, control experiments *Fla*$^+H_1{}^b$—x *Fla*$^-H_1{}^b$ also showed inhibition by *i* serum, which must therefore be attributed to a delicate cross-reaction between *b* and *i* (not observed in agglutination tests or in inhibition of swarms, and not necessarily a flagellar reaction, cf. LEDERBERG and IINO 1956, ¶3.14) rather than the necessary presence of the *i* antigen on the motile initials. As a comparable non-*b* *Fla*$^-$ stock is not available the specificity of inhibition by *b* serum has not been verified.

2.19 Other serums (*a*, *c*, *d*, *k*, *r* and *1.2*) were then tested on the *b*—x *b* controls, and all were found non-inhibitory, in contrast to *i* and *b*. Further experiments were therefore conducted with the transductions *Fla*$^+H_1{}^a$ (SW-940)—x *Fla*$^-H_1{}^b$. With this system, all initials were completely inhibited by 1:1000 *b* antiserum (figure 11) and were all also partially or completely inhibited by anti-*a*. In microclones all cells (except a few which generated *b* swarms) were slowly but completely immobilized by *a* serum. In dilute NGA + *a* serum (see 3.3) stationary colonies and a few short trails were seen (fig. 12). This result is evidence, not otherwise available, of the homogeneity of the exogenotes, i.e., that each one that carries the *Fla*$^+$ marker also carries the coupled H_1. The occurrence of recombinant clones *Fla*$^+H_1{}^b$ may therefore be attributed to crossing over in a transient, initial heterogenote *Fla*$^-H_1{}^b$/ex *Fla*$^+H_1{}^a$ (cf. MORSE *et al.* 1956b; DEMEREC and DEMEREC 1956).

2.20 Intermediate motile isolates from multilinear clones have also been tested separately with *b* or *a* serum. Most were inhibited but in one test, two of four cells isolated were unaffected by *a* serum, and may have had a pure *b* serotype. These terminal non-*a* lines may account for the residual trails seen in NGA platings with this antiserum.

2.21 A search was made for reciprocal crossovers, i.e., *Fla*$^-H_1{}^a$ among nonmotile progeny in multilinear clones and co-segregants (2.22) in clones containing motile transformations (both $H_1{}^b$ and $H_1{}^a$). These are detected as isolates capable of yielding motile, non-*b* recombinants when tested x—*Fla*$^+H_1^b$ in NGA plus *b* antiserum. None were found in a total of about 100 tests.

2.22 *Clones with swarms: segregation.* As stated, 2.3 and table 2, about four percent of motile initials gave clones which contain ten percent or more motile individuals. These have also been replated on NGA and verified to initiate swarms. Several dozen individuals from these progenies have also been allowed to form clones and proved to be regularly motile, without continued segregation. They therefore correspond to stable transductions of motility. However, the initial clones are often mixtures of

stably motile and stably nonmotile cells, shown in terms of proportions of motile to total cells:

Pure +	~½ +	~¼ +	~⅛ +	<⅛+
16	8	3	7	2

These data include microclones observed directly as well as those plated in NGA which gave swarms 3.12. The inference that many of the clones are segregating is supported by pedigrees on five additional isolates in which segregation was observed directly until not less than n_1 (two cases), n_2 (two cases) and n_4 (one case). One of the n_1 pedigrees, already cited in 2.16 generated twenty-two lines in a cosegregant of the swarm. Only two other pedigrees (which segregated swarms to at least n_1 and n_2 respectively) were directly informative on the number of lines generated by co-segregants (non-swarm sibs) of swarm-producing cells. The numbers were zero and one respectively (cf. 3.12).

3. *Plating experiments*

3.0 *Recapitulation.* Motile initials and single clones therefrom were plated in soft agar, and the correlation of motile individuals and lines with trails was verified. However, the fraction of motile cells which formed trails in agar depended on the stiffness of the medium, and no generalization could be made on the distinction of linear from multilinear cells by this method. The general features of the clones, already stated from pedigree experiments, were reaffirmed in the plating experiments. Several trails might be generated by a single initial. The early segregation of motile and nonmotile holoclones was verified, as was the occurrence of motile holoclones (swarms) as sibs to multilinear subclones.

3.1 *Correlation of motile initials with trails in NGA.* It has so far been plausibly assumed that motile lines correspond to visible trails of colonies in NGA. However, experiments to verify this correspondence were at first indifferently successful. Motile initials were pooled after isolation from trapping droplets (2.1) and transferred individually to fresh drops. These were then taken up, one at a time, in a hand-controlled quartz pipette (LEDERBERG 1954) and blown out on to the surface of an NGA plate. By this technique of blind transfer (which is, however, much less laborious than the more certain procedure of transfer by the micropipette used for manipulation) somewhat more than half the isolations were successful in terms of outgrowth at the point of transfer. Thus, 29 motile initials planted on NGA gave 18 outgrowths: three were swarms, the remainder were stationary colonies or clusters of two or three fused colonies. No trails were seen.

3.2 As it was supposed that chemotactic influences (see 4.11) might encourage motile bacteria to remain at the surface layer of the agar, trials were made of plating motile initials in deep NGA. Pools of initials were collected and ejected from the micropipette into 0.5 ml broth. Samples of the diluted pool were than made up to contain 100 or 200 cells and plated with 10 ml molten NGA in 6 cm Petri dishes, or 25 ml NGA in 10 cm Petri dishes. The plates were then chilled to set them promptly

and incubated overnight at 37°C. All too often, the readings were already somewhat obscured by overspreading swarms, but it was usually possible to enumerate swarm centers as well as colonies and trails. As an arbitrary classification, a *trail* is an aggregate of ten or more microcolonies in NGA; a *cluster* has from two to ten; a *colony* (self-evidently) just one, and a *swarm* is a diffuse cloud of growth. These units, taken together, are *viable centers*. The summation of a number of platings from several preparations in which about 900 initials had been plated is:

	Colonies	Clusters and trails	Swarms	Viable centers
Number	732	86	31	849
Per cent	86	10	4	

3.3 This incidence of swarms is comparable to the yield in table 1; it is quite clear that only a small proportion (here ten percent) of the motile cells plated can form trails in NGA. However, considerable variability in the proportion of trails was noted from one day to the next. This variability was traced to the fluidity of the NGA, which is poorly reproducible unless special care is taken to disperse all of the gelatin and agar evenly. It was found that the addition of progressive volumes of broth, to dilute the NGA, would give progressively higher yields of trails, while concentrating the medium had the opposite effect. For example, aliquots of the same pool of initials were plated, with the following results.

NGA diluted with broth	Colonies	Clusters	Trails	Swarms	Viable centers
—	53	2	2	2	59
4:1	46	5	6	0	57
7:3	39	18	11	3	71
3:2	19	11	19	4	53

Clearly cells that appear only as compact colonies in NGA (fig. 7) are manifest as clusters and trails when the medium is diluted (fig. 8). The last medium (dilute NGA) was adopted as the softest that could be cleanly handled without slopping (except in midsummer).

3.4 The platings of well-separated initials show "trails" which are no longer strikingly linear, especially in dilute NGA. At least part of the rectilinearity of the trails formerly figured may be attributable to the orientation of a chemotactic gradient of metabolic products from the excess recipient cells (cf. 4.11). The isolated trails may more closely resemble the tracings of random walks but their own appearance, together with that of seeded NGA under the microscope suggests that the progressive movement of motile cells in this medium is confined to microscopic interstices in the gel. These interstices may also entrap motile cells, which would account for the appreciable proportion of initials which give no trail at all; even when NGA is seeded with fully motile clones, only a minority of the cells are visibly motile at any one time.

3.5 The rectilinearity of trails is, of course, also complicated by the genetic multi-

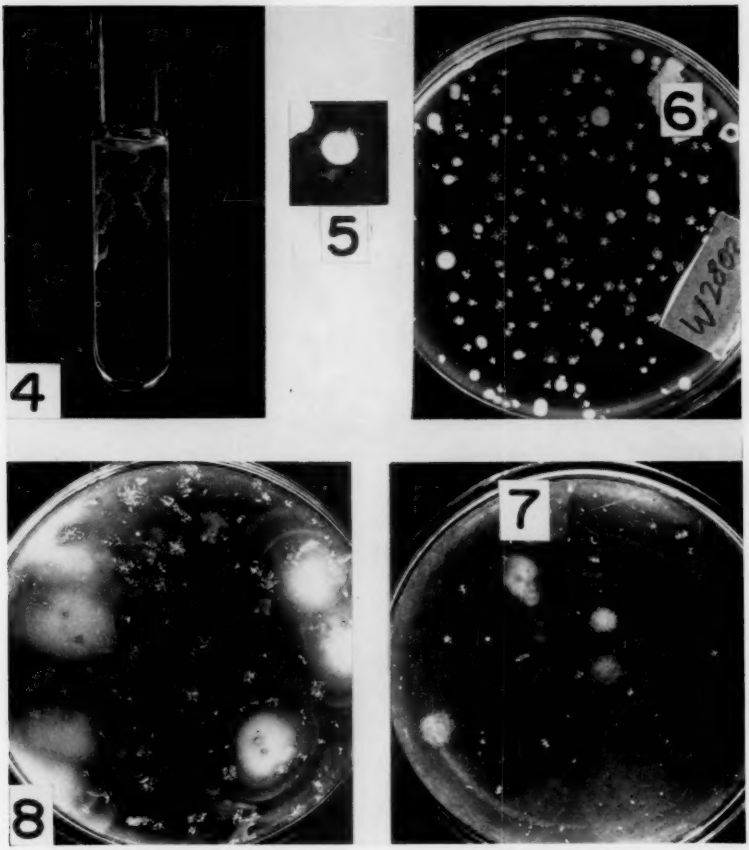


FIGURE 4.—Unusually long trails from SW-553 x— SW-666. The transduction mixture was diluted and samples planted at the top of a series of NGA tubes. Many of the tubes showed swarms, which appear as cylindrical clouds which gradually progress to the bottom, as well as trails. This tube lacks swarms but shows two trails of unusual length. The tube was incubated several days before being preserved for photography, and some irrelevant growth at the glass-agar interface is also evident. This and other figures approximately life size unless indicated otherwise.

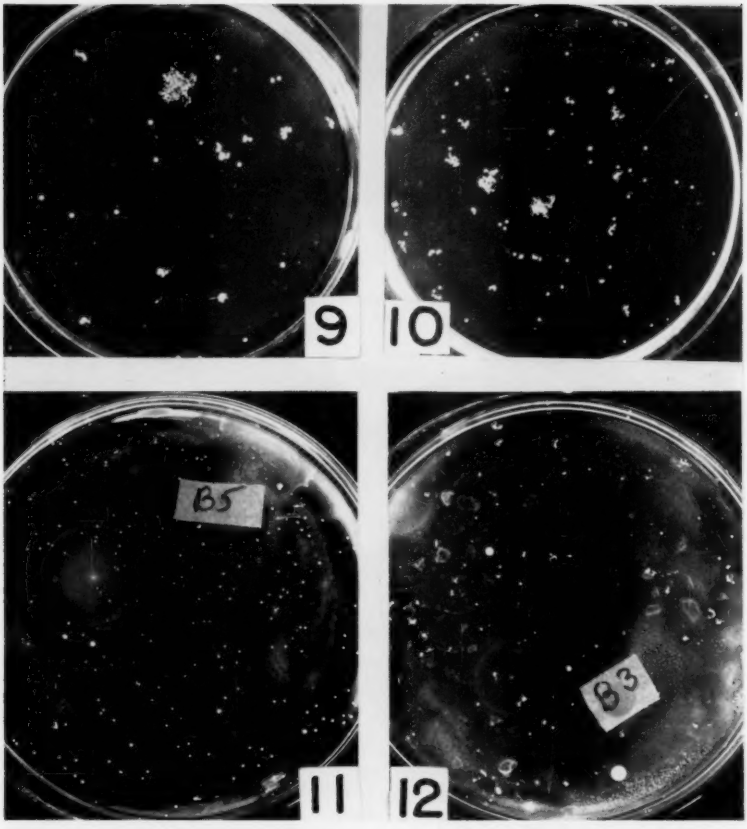
FIGURE 5.—A colony of SW-553 in deep NGA showing a spontaneous trail. About 3×.

FIGURE 6.—*E. coli* mutant W-2802 showing abundant satellites, in deep NGA.

FIGURES 7–8.—Pooled initials from SW-666 x— in NGA and dilute NGA respectively.

linearity of the clones produced by many initials. A few platings have been made of intermediate motile cells, isolated ca. n_{10}. The trails these cells produce are generally less profuse than those made by initials and they may sometimes assume a definitely rectilinear aspect, though the presence of doublets is again remarkable.

3.6 The occurrence of swarms in these platings has also been noted. Owing to



FIGURES 9–10.—Single clones of SW-666 x— from motile initials showing several large and small trails each. Plated in dilute NGA.

FIGURES 11–12.—Pooled initials of SW-666 x— SW-940 in dilute NGA containing b and a antiserum, respectively. Compare with figure 8.

overgrowth, observation of swarm centers has been difficult, but in favorable plates, about half the swarms are notably centered by a compact colony or a trail, indicative of early segregation as the counterpart of 2.21. This centering was not observed when motile clones were replated, and should not be confused with "flares" 4.6.

3.7 *Platings of clones.* The technique of 3.1 was used to transfer single initials to .5 ml volumes of broth. These were then incubated about three hours, and plated into NGA. The plates were then incubated overnight. Altogether, 282 isolates were transferred, 186 successfully. The clones averaged about 2^6 viable centers each, but showed considerable variation, presumably because of a high dispersion of the duration of lag (cf. CAVALLI and LEDERBERG 1956). For example, the following clone

sizes were noted in one series of parallel platings: 7, 8, 10, 17, 21, 23, 25, 30, 32, 44, 44, 54, 65, 66, 69, 73, 81, 87, 101, 106, 109, 140, 142, 143, 149. The non-integral powers of 2 also illustrate early dissynchrony, though this may be exaggerated by the temporary staying together of essentially divided cells. About five percent of the viable cells are not recovered in the plates, as was shown by counts in deep agar added to the residue in the broth tubes after plating. These variables indicate the need for caution in postulating statistical homogeneity in bacterial growth.

3.8 Of the 186 viable clones that were plated, 155 showed only compact single colonies, or occasional doublets of doubtful significance, precisely as did platings of clones of control SW-666 cells. Sixteen clones included one trail, three clones had two and one (possibly in a soft batch of NGA) had ten trails in addition to compact colonies. The three or four exceptional clones indicate that the potentiality of forming a trail even in NGA is not a strictly unilinear legacy (cf. 5.3).

3.9 The remaining 11 clones included swarms which usually spread so that they could not be counted. Three of these were pure; 8 had colonies or trails as well, and are therefore included as examples of segregation in 2.22.

3.10 After the effect of diluted NGA was discovered (3.3), 62 initials were processed for the plating of clones in this medium. As expected, the incidence of trails was much higher than in the preceding experiment. Thirty-nine viable clones included 22 with trails (see figs. 9, 10), 15 with single compact colonies only, and two with swarms, both segregating. The following numbers of motile lines (trails and clusters) were found in this series of clones: fourteen zeros, and 1, 1, 1, 2, 3, 3, 4, 6, 8, 8, 9, 11, 11, 11, 14, 15, 17, 20, 20, 21, 21, 25. As compared with table 1 this distribution may be shifted slightly to the right, which is expected because motile lines that terminate before n_{10} would be missed in microclones, but may well be detected in dilute NGA.

3.11 A few clones were also processed from intermediate isolates. In accord with 2.6 these gave at most one trail.

3.12 In five of the thirteen swarm-clones of these experiments, the numbers of cosegregant trails could be counted for comparison with 2.21 as follows: 2, 3, 5, 18, and 20. The other five segregating swarm-clones either had compact colonies or, if trails were present they were obscured by the swarm. As already noted, three of the swarm clones were pure.

4. *Miscellaneous observations*

4.1 *Experiments with SW-553; spontaneous lines.* SW-553 is, as previously described (LEDERBERG and EDWARDS 1953) a nonmotile *Salmonella dublin*, *Fla*$^-H_1^{gp}$. Unfortunately, many experiments which later had to be repeated with SW-666 were first conducted with SW-553. This stock was initially chosen because its *Fla*$^-$ mutation, although distinct from that of SW-666 (STOCKER *et al.* 1953) is also linked to H_1. Furthermore it gives spectacularly long trails (fig. 4) when motility is transduced to it from other strains. However, it has also been found to produce trails spontaneously, though these are always insignificant when compared to those obtained by transduction. This effect was first noticed in platings of clones from motile initials (cf. 3.7) when every colony in some plates was observed to have a small

satellite trail (as in fig. 5). The same effect was then observed in control platings. It can be discerned regularly only in dilute NGA, which accounts for its having been overlooked previously. SW-666 did not give this effect in comparable platings, and was therefore adopted in place of SW-553 for further studies. Otherwise, the general features of the transduction experiments with SW-553 were quite similar to those reported in 2. and 3.

4.2 When it was learned how to trap motile initials from concentrated suspensions (2.1), the procedure was applied to untreated cultures of SW-553, and motile initials were found about one thousandth as frequently as from transductional mixtures. These were isolated and found to generate lines which petered out after a few divisions, which, together with the appearance of their minute trails in agar makes them comparable to the late lines in transductional clones (2.7).

4.3 Other *Fla*$^-$ cultures which produce spontaneous trails more frequently have been noted (STOCKER *et al.* 1953) and are being studied more systematically (QUADLING, cited by STOCKER 1956a).

4.4 A similar appearance, with more profuse satellites, has been noted in a number of mutants which were recovered in so far unsuccessful attempts to obtain absolute nonmotile mutants of *E. coli* K-12 (fig. 6); the patterns of transmission of motility in this material have not been investigated in detail but are seemingly quite irregular. In general, *E. coli* is not as aggressively motile as are most Salmonellas.

4.5 *Delay of expression in motile clones.* When cells from motile clones of Salmonella were observed at fission, both daughters were promptly and equally motile. Salmonella is, however, characterized by numerous flagella per cell, so that this result is not inconsistent with a latent polarity. LEIFSON (1951) has described bacteria with unipolar flagellation in two categories, based on his observations of stained smears: those in which incipiently dividing cells already show a flagellum at each (distal) pole, and those in which only one flagellum is apparent. The second situation would be attributed to a relative delay in the formation of a new flagellum after the onset of fission. Through DR. LEIFSON's courtesy, a culture of this type (*Pseudomonas aeruginosa* H1A) was available and preliminary observations on living material have been made. The most extensive pedigree is depicted in figure 1D, where + and − refer to apparently motility of a cell at the moment of its separation from its sib. Thus, every cell transmits motility to its progeny. However, a + initial has one +, one − daughter; a − initial two + daughters. This rule can be rationalized if a flagellate cell makes no more flagella, while its daughter, *née sans* flagella, makes two *de novo* prior to the next fission. As is also perhaps to be expected, occasional exceptions to this rule were found in other more fragmentary pedigrees, and more work will be needed to validate the pattern on a sound statistical basis.

4.6 *"Flares".* STOCKER *et al.* (1953) noted that the centers of transductional swarms often contained an accumulation of denser microcolonies, very variable in size. At that time, it was wondered whether these might represent a segregational process, but no nonmotile derivatives could be isolated from these "flares" (the actual center of the swarms being crowded with excess recipient cells). It is now apparent that the flares have no special relationship to the transduction process, as they have been seen on replatings of motile clones, both of transductional and stock culture origin.

They are especially prominent in platings of rough Salmonella strains and of motile *E. coli*, and are presumably related to autoagglutination. Selection for rapid motility in motile clones, or of the smoothest colony forms in nonmotile recipients, minimizes the flares which are produced on direct plating, or in transduction experiments, respectively.

4.7 *Applications of viscous adjuvants* (*Methocel*). Through the courtesy of The Dow-Corning Company, Midlands, Michigan, Methocel (methylated cellulose) preparations of various viscosity types were available for these experiments. Methocel 4000 (the viscosity in cps of a two percent aqueous solution) made up in broth and kept in microdroplets in an oil chamber was found to keep the daughter cells together after bacterial fission, so that intact chains of 2, 4, 8, to as high as 128 and 256 cells are formed. These are useful as a simple means of detecting certain relationships in cell lineages. For example, cells with a polar formazan granule generate chains in which the granule is still regularly observed at a polar position in the terminal cell. A few percent of the stained cells carried the granule interstitially and correspondingly generate chains with the granule in an interstitial cell (usually at a polar position). The formazan granule is thus inherited linearly, each division along the line giving one Fz^+ and one Fz^- cell. The formazan is evidently metabolized by Samonella cells, as the granule gradually fades and disappears in the course of five to ten fissions.

4.8 The regularity of these chains makes them sensitive indices of liminal amounts of multiplication and death, which are especially difficult to detect if they are concurrent (cf. discussion by Ryan 1955). Multiplication is revealed by the increment of two- and four-celled chains in an initially dispersed suspension, and death by gaps (in which faint ghosts can sometimes be made out) in the chains. Chains allowed to develop for three to six fissions and then held for a day or two in the refrigerator were found to suffer about one percent of deaths; however these were not correlated with each other, with formazan granules or either end of the chain, and are therefore considered as indeterminate events, rather than consequences of "aging" or genetic damage.

4.9 After preliminary trials, Methocel 400, one and one-half percent in broth was found to give a threshold level of viscosity that barely permitted most of the cells of a motile clone (from a previous pedigree isolation, and not selected for augmented motility) to continue moving. Large pools of initials were placed in droplets of this medium, but no initial cells were found whose motility (during the first three hours after the transduction mixture was made up) approximated that of the motile clone. Later, motile swarms overgrew the drops as expected. Clearly the full development of the motility phenotype of a Fla^+ clone requires considerably longer than its first manifestation. About one percent of the initial cells gradually crept into the methocel in an hour's time, but these cells did not consistently generate a higher fraction of swarms or multilinear clones than unselected samples of initials. These experiments were hindered by an undue wetting of the cover-glasses by the methocel solutions, so that the drops eventually spread and mixed with one another. Attempts to discriminate future swarm- or multilinear clones by the maximum persistence of activity of the initials for one or two fission intervals in ordinary broth were equally inconclusive.

4.10 Trials were made on the incorporation of methocel to regulate the viscosity of agar, but flocculent precipitates and poorly gelling media resulted.

4.11 *Chemotaxis and bacterial behavior.* There is an extensive literature on chemotactic behavior in bacteria but as most of it is fifty or more years old (cf. JENNINGS 1906) it is no longer often quoted. As controls on experiments 2.19 the agglutination of motile clones by homologous antisera was followed microscopically. Paired droplets of bacteria and serum (1:100 and 1:1000) were arranged on a cover-glass over an oil chamber in preparation for mixing. After several drops had stood for about ten minutes it was noticed that the motile bacteria had accumulated at the distal portion of their drops, leaving the proximal portion almost free of cells. The two drops were separated by several mm. of oil, and the accumulation of cells was readily seen by the naked eye. The experiment was readily and reproducibly repeated, but not so readily understood. However, it was found that the negative taxis did not depend on the specificity of the serum, but that only preparations issued by the Central Public Health Laboratories, Public Health Laboratory Service, London, England, showed the effect. It was then realized that these preparations were preserved with five-tenths percent cresol, and this substance (or phenol in its stead) was then inculpated as the tactic stimulus. Minute amounts of phenol (from a source which may be only 10^{-5} M to start) evidently can dissolve and diffuse through the mineral oil to the bacterial drop and establish a concentration gradient in which the bacteria seek the lowest level. The behavioral basis for the migration (cf. JENNINGS 1906; ROTHSCHILD 1956) is not obvious, but is more likely to depend on comparisons at points along the path of the cells, rather than on a continuous vectorial orientation of the cells along the chemical gradient, as no such orientation was observed. At the sites of accumulation, the cells showed very rapid but jerky movements (i.e. a short mean free path) as compared to their initial behavior and this may account for the accumulation, without offering a clue as to how the chemical information (at such low concentrations) is transmuted into behavioral response (JENNINGS and CROSBY 1901). The levels of phenol employed here are quite innocuous to the viability of the organisms. The phenol effect could not be obtained with *E. coli* nor, (it goes without saying) with nonmotile strains. Attempts to quantitate the response have been hindered by its failure to occur, at least in such spectacular fashion, in capillary tubes where concentration gradients and rates of migration could be accurately controlled and measured. A somewhat similar behavioral response is evoked by staled medium (regardless of pH). In trapping procedures, motile initials have been noticed to accumulate to some degree in the zone at the margin between the mass culture and the empty drop, rather than occupy the latter freely, which is a slight hindrance to fishing for them with a micropipette.

4.12 *Position effect?* At least five distinct *Fla*$^-$ mutations each linked to H_1 have been recorded (STOCKER *et al.* 1953; LEDERBERG and IINO 1956). The following four have been tested in all transductional combinations: SW-666, SL-28; SW-553 and SW-1157. (The fifth, SL-13, poses certain technical difficulties as it responds very meagerly to the phage and cannot be used as donor.) In every combination, not only swarms but also trails were observed, which indicates that these mutants though commonly linked and affecting the same character do not show a cis-trans position effect (cf. 5.6 and MORSE *et al.* 1956b).

5. DISCUSSION

5.1 Three principal topics will be discussed: evidence for and material interpretation of linear inheritance in Salmonella, analogous examples of asymmetric cell division, and their significance for inferences of biological particles. Because more than one interpretation can be put on the data of this paper, they have been left as bare as possible up to this point. Two aspects of linear heredity are in question: the linear propagation of motility from intermediate isolations, in which linear inheritance is directly observed, and the possibly linear propagation of "*E*" which can be inferred as indicated below, 5.3.

5.2 *Motility lines.* These require little discussion as extensive pedigree 2.7 and some plating analyses 3.5, 3.10, 3.11 leave little doubt of uncomplicated, linear transmission of motility in many *sub*clones.

5.3 *Linear partition in multilinear clones.* Contrary to first expectation STOCKER *et al.* 1953) motile initials often generate more than one motile line. The quality *E*, of generating many motile lines, is partitioned very unequally at fission (fig. 3). Does *E* constitute a line? The distribution (table 1) suggests that about ten lines might bound "few" from "many", and only one point on figure 3 (perhaps a statistical overlap) shows both sibs to have generated more than ten lines each. However, *E* has not often been followed in pedigrees beyond the first few divisions, so that the duration and singularity of the postulated *E* line would not have strong evidential support. Furthermore, several clones contained both *E* and motile subclones. With these reservations (see also 6.2) the present experiments are indecisive but not grossly incompatible with the hypothesis that *E* is linearly inherited.

5.4 *Material equivalents; particles and lines.* Linear inheritance conveys a compelling suggestion of a discrete, nonreproducing particle which is passively inherited by one offspring of a dividing cell. It is, furthermore, plausible to correlate the motile line with a motility-conferring particle, a flagellum or its Anlage, a blepharoplast. There is no direct evidence for this identification, which might be helped by electron-microscopic descriptions of unilinear cells as uniflagellate. However, for the sake of further argument this hypothesis will be tentatively adopted. Nevertheless it should be cautioned that the immediate basis of linear inheritance is recurrent, asymmetrical division, and that this can be accomplished in ways in relation to which the hypothetical particle would be a remote abstraction.

5.5 The correspondence of *E* with a particle is, for its part, independent of whether its transmission is strictly or almost linear, as the laws of distribution of the particle can readily be accommodated to fit either experimental result. Many of the experiments in this paper are therefore, from this point of view, directed to a secondary issue. The following hypotheses are stated for the strict linear case; the qualifications needed to suit them for occasional deviations are obvious.

5.6 The termini of these experiments are fairly clear: we begin with a transduced exogenote and end up with a flagellum. We can therefore ask two questions of *E*, which are not necessarily logically related: what is the relationship of *E* to each of these elements. To give hypothetical answers for the latter first, we may postulate:

A. *E* is a bundle of flagella, or of units bearing a unitary relationship to them. *E* therefore divides (but does not multiply) in a requisite asymmetrical pattern.

B. *E* is a catalyst or precursor of flagellar formation. (These alternatives cannot be distinguished without stoichiometric data on flagellar synthesis.)

Some answers to the other question are:

1. *E* is the exogenote which has failed to exchange with the recipient chromosome in a reproductively efficient fashion and therefore fails to reproduce. Its present location, and the nature of the accident that led to the abortion are unspecified.

2. *E* is "products of gene action". These may have accompanied the exogenote in transduction, or may have been synthesized in a transient heterogenote when the exogenote first inhabited the recipient cell. They result in motile lines when they are left in cells from which the exogenote has disappeared or segregated.

The combination of (A,B) with (1,2) leads to four alternative, tenable versions of which the following models are suggested as plausible representatives:

A1. A polytenic exogenote is transduced and, failing to be incorporated, is sorted out among the progeny of the initial cell. Each unitary chromonemal segment makes (or is converted into) one flagellum. The bundle of chromonemata tends to splinter rather than divide equally at cell division.

A2. An exogenote is transduced and begins to function in the recipient cell regardless of the success of incorporation. Consequently numerous flagella are formed at an active pole of the cell. The exogenote may then disappear, or segregate into a sib cell. The flagella are no longer synthesized, but are asymmetrically sorted out among the progeny, thus generating a multilinear clone.

B1. An exogenote is transduced and, failing to be incorporated, persists as a functional, nonreproductive particle. It therefore mediates the formation of flagella in the line of cells which carries it. The flagella are sorted in the progeny of the progressive sib clones.

B2. An exogenote is transduced and mediates the formation of a flagellum-synthesizing system or organelle. This system is transmitted linearly and continues to function after the exogenote is lost from the cell which carries it.

5.7 For exceptions to the unilinearity of *E*, we can simply postulate that the exogenote (under 1) rarely multiplies or (if initially ditenic) divides; under 2, the exceptions would reflect the imperfection of polarized division of the cell.

5.8 The data of the present paper do not permit a critical choice among these alternatives. For A versus B we should need more knowledge of the chemical and morphological pathways from gene to flagellum. For 1 versus 2, a conceivable experiment may be possible with increasing knowledge of the linkage map of Salmonella, and the availability of two genetically linked but physiologically unrelated markers that could be diagnosed in linear inheritance. If the markers remained linked in lines, we would either have to question their physiological unrelatedness or admit their association in lines on a genetic basis, which would be tantamount to 1, the hypothesis of the the residual exogenote.

5.9 The experiments on the persistence of H_1^a action and 2.18 may be considered as models of the proposal, but this marker is too closely related in its action to *Fla* to make a convincing argument. Thus, the persistence of the *a* antigen may mean no more than that an antigenically differentiated intermediate has been formed in a cell which initially carried H_1^a as well as H_1^b.

5.10 Finally more knowledge of bacterial growth may bear on the plausibility of asymmetrical fission via a system other than a discrete particle. Meanwhile, we may have to admit that this material gives us no means of differentiating the action of a gene from that of its intermediaries, as long as both are so hypothetical.

5.11 *Segregation of swarm-clones.* The fact that swarm clones are often mixed with *Fla*$^-$ 2.22 might be hoped to throw some light on the mechanism of incorporation of transduced exogenotes. However, it has an almost trivial explanation in the multinucleate character of enterobacterial cells, and similar early segregation has been recorded in experiments on mutational (LEDERBERG 1949; WITKIN 1951; NEWCOMBE 1953; RYAN and WAINWRIGHT 1954) sexual-segregational (LEDERBERG 1956b), lysogenizing (LEDERBERG and LEDERBERG 1953; LIEB 1953) and other transductive (MORSE *et al.* 1956a) systems. The variation in extent of segregation may also depend on the truncation of early pedigrees 2.1 and on a variable delay before incorporation occurs. These variables make it impossible to lay any weight on segregation as evidence for the copy-choice versus direct replacement hypothesis of transductive incorporation (SYMPOSIUM 1955, HOTCHKISS 1956). It has already been emphasized that incorporation of a marker involves the displacement of its homologue, not merely an addition to the genotype, as can be shown particularly with antigenic markers.

5.12 In the transduction of *Gal* loci in *E. coli* (MORSE *et al.* 1956b) segregation can be delayed indefinitely. That is, a heterogenote is formed in which the fragment can proliferate coordinately with the cell, before segregation occurs sporadically. It is therefore readily shown that incorporation involves crossing over, in the sense that a variable part of a given exogenote appears in the progeny of different segregation events. In Salmonella, the heterogenote initially formed by transduction evidently cannot persist and incorporation and segregation of the exogenote must follow promptly on the injection of the phage. It has previously been noted (STOCKER *et al.* 1953, and is now corroborated in the material of 2.22) that no clones contained two serotypes of swarms, which is a further argument for the transiency of the heterogenote stage. However, the uniform coupling of *Fla*$^+$ with $H_1{}^a$ in motile initials 2.19, and their separation in *Fla*$^+H_1{}^b$ swarms support a cross-over model of incorporation, similar to that which is more directly inferred for *E. coli*.

5.13 *Asymmetry in cell division: bacteria.* Enteric bacteria appear to be simple rods, lacking head-tail asymmetry. Other bacterial species are more clearly differentiated, spectacularly in Caulobacter which has a long stalk, terminated by flagella at one end (HOUWINK 1955). At cell division, the body of the cell is split, concurrently with the development of a flagellum and ultimately a stalk at the distal (free) pole. These observations also put in question the possibility of genetic continuity of the flagellar Anlage, (BISSET 1956) unlike the division processes described in many protozoa. Bacteria with unipolar flagellation, like those studied by LEIFSON (1951) pose the same problem of the morphogenetic relationship of the old and new flagella. At any rate, each fission of such a cell may be considered to engender one new cell (in respect to the flagellum) and leave one old one. Whether the sequence of oldest cells in a series of fissions will constitute a detectable line can be thought of as depending on how completely a mature flagellar apparatus is resynthesized by the young cell in an inter-

fission interval. The motility lines would represent the extreme case where no such synthesis is possible owing to genetic defect; intermediate situations are represented by spontaneous trails 4.1, and by the observations on *Pseudomonas aeruginosa* 4.5 and *E. coli* 4.4.

5.14 The overt symmetry of Salmonella and other bacillary species may then be only superficial; suggestions of head-tail or corresponding mother-daughter differences come from the unipolar deposition of formazan 2.14 and the basophilia of one or both poles, interpreted by BISSET (1956) as indicative of a growing point. Differences in the reaction of sib daughter cells to staining procedures (PENNINGTON 1950) and to antibiotics (LINZ 1954; SMILES, WELCH and ELFORD 1948) are also indicative of unequal division. However, these scattered observations have not yet been correlated to the extent that this conception of the organization of the bacterial cell may be regarded as more than a speculative working hypothesis.

5.15 *Protozoa.* Outside the bacteria and simpler algae, very few unicellular organisms divide by simple fission, so that there are many opportunities for asymmetric division and linear transmission. For example, JENNINGS (1908) and MCCLENDON (1909) have described the linear inheritance of accidental pellicular defects for as many as twenty-two fissions. This pattern is a simple consequence of the conservation of the surface structure of the Paramecium at cell division. In a related experiment, JENNINGS (1937) injured the teeth of Difflugia by surgery and found that both daughters of an injured individual were often defective. The deviation from linear transmission in this case is apparently due to the role of the teeth of the mother cell as models for the deposition of the daughter's. In an appropriate context, then, silica can simulate a gene. However, successive offspring from the line of cells carrying the original defect were progressively more normal. The experimental defect therefore did not generate a defective clone, but one with a variety of defective lines. In all of JENNINGS' experiments, the morphological description of the propagation of variations averts the postulation of discrete particles.

5.16 *Algae.* The algae furnish several examples of lines which depend on peculiarities of cell division. A desmid, for example consists of two modified hemispheres, "semi-cells" joined by a narrow isthmus in which the single diploid nucleus is lodged. At cell division, the nucleus undergoes mitosis and the semi-cells separate, each one later budding a new semicell to form a new cell. WARIS (1950) and KALLIO (1951) have described mutations in Micrasterias which seemingly have a cytoplasmic basis, since the progeny of a defective semi-cell remain defective, and of the normal, normal, despite the common nuclear origin. In one case, a reversion was described which resulted in a cell with one normal, one mutant semi-cell. This "dichotypic" initial generated a clone in which all of the new semi-cells thenceforth were normal. The clone was thus unilinear for dichotypy: the equivalent "particle" in this instance may be the whole cytoplasmic architecture of the differentiated, mutant semi-cell which persists after the genotype is altered by mutation.

5.17 The grov h of diatoms also generates lines of a sort. The cell is bounded by two half-shells which fit into one another like the lid and base of a Petri dish. In many species, the walls are so rigid that they limit the growth of the daughter cells after division. Therefore, the daughter that receives the *inner* half-shell of the parent.

and uses this for its *outer* half-shell, is regularly smaller than the other daughter, which maintains the same size as the parent. Each clone therefore contains only one line of cells as large as the parent, and a series of lines of progressively smaller size. The mean cell size of a clone diminishes progressively with time, but can be restored by the formation of (often sexual) auxospores in which the rigid walls are discarded. As the cells become smaller they lose the capacity for auxospore formation and ultimately for vegetative division too, and the clones are therefore doomed (WIEDLING 1948; cf. RIZET 1953). A formal scheme of progressively attenuated particles might have been constructed for this finite longevity, but again is less meaningful than the inference from direct observation of the division mechanism.

5.18 *Yeast.* Two types of lineation are possibly related to the proliferation of yeast by budding. The disproportionate partition of total cytoplasm may account for the lines of normal mother cells, with small-colony-variant buds, described by EPHRUSSI and HOTTINGUER (1951) in experiments with acriflavine. BAUTZ and MARQUARDT (1954) have indicated that anaerobic, Nadi-negative mother cells placed in air remain negative but form positive-buds. On the other hand, SPIEGELMAN (1951) concluded that the cytoplasm was equally divided between mother and bud from an analysis of another cytoplasmic character. If both observations are correct, it may be necessary to assume that acriflavine actively influences the segregation of the postulated plasmids, perhaps by aggregating them.

5.19 A second effect of budding is to leave a "birth scar" on the bud, and add a bud scar to the wall of the mother. Each clone should therefore continue a line of the oldest cell, carrying the most scars. Since a new bud was never observed to form at the site of an old scar, the oldest line, indeed any line, presumably has a finite lifetime, (BARTON 1950, BARTHOLOMEW and MITTWER 1953), though this might be up to one hundred buddings; twenty-three were observed by BARTON. Remarkably, BARTHOLOMEW and MITTWER reported having found one cell with twenty scars: its incidence in the entire clone would be calculated at 2^{-20} or one in four million.

5.20 *Morphogenesis.* Morphogenetic differentiation must always be related to unequal division in the long run. When divergent clones are produced, the process resembles segregation; however, stem cells of various kinds (e.g. apical cells in shoot meristems; teloblasts in annelid embryos) form approximations to lines, although the detailed cell linages have not often been worked out. In the renewal of spermatogonia in the rat, however, CLERMONT and LEBLOND (1953) have secured evidence that the ancestral spermatogonia undergo cycles of fission whereby one daughter (or son?) proliferates and differentiates into eight or sixteen spermatocytes, and the other remains an undifferentiated stem cell. The material foundation of the line of stem cells is unknown.

5.21 *Orthoclones and senescence.* The line of individuals of a given age in a series of progenies has been designated an "orthoclone" by LANSING (1948) who has also described progressive changes in old orthoclones of rotifers.

5.22 The relationship of linear inheritance to aging mechanisms may be conceived in terms of individual cells of the line (or orthoclone) or to the average properties of the clone. Thus, SONNEBORN (1930) demonstrated that the line of head-bearing individuals on repeated fission of the worm Stenostomum has a finite life time, while

the line of tail segments can be propagated indefinitely. The aging of a head may be related to the concentration of differentiated structures at that end of the worm; tail segments regenerate new (i.e. "young") heads at fission. Attempts to demonstrate a similar aging process in bacterial cells 4.8 and in yeast 5.19 have been unsuccessful, but have been carried for only a limited number of generations. The maternal orthoclone in yeast should ultimately show the effect of progressive scarring.

5.23 On the other hand, if we define aging as the progressive loss of a function in a clone, we should consider the progressive dilution of the parental character as any uni- or multi-linear clone increases. Thus an initially motile individual of Salmonella generates a clone in which the average motility tends toward zero, and reaches it with the occasional termination of lines. The aging of a diatom clone is a parallel but more obvious example. In these examples, however, the parental line is itself the most juvenile element of the population, in so far as it most closely resembles the initial state.

5.24 *Chromosomes.* Recent studies on the material (in contrast to the informational) content of chromosomes and of the deoxyribonucleic acid of phage have raised the question whether mitosis is ever equal. The Watson-Crick model of DNA structure (CRICK 1954) postulates two complementary polynucleotide helices, which might separate at mitosis and generate the alternative complements. Recent experiments by LEVINTHAL (1956) are consistent with this semi-conservative model of replication of phage DNA: after the initial separation, each helix is conserved as such and the material content of the original DNA will be found as two lines in a holoclone carrying its genetic information. Analogous studies by MAZIA and PLAUT (1955) on Crepis chromosomes indicate a single line, as does the suggestion of tinctorial differences between "mother and daughter chromosomes" (PROKOFIEVA-BELGOVSKAIA 1946; cf. MULLER's criticism appended thereto). It is therefore plausible to look for linearly transmitted modifications that have a chromosomal basis.

5.25 *Particles.* The axiomatic foundation of genetic analysis is the particle. However, the term should not be taken too literally, especially when applied to a mathematical rather than a material entity. Particulate forms of inheritance apply to such amorphous items as acreage, titles and protoplasmic lumps, from which we can infer that a *particle* is essentially a rule of division, which can be stated in physical, legal, or biological terms, and whose domain of validity must not be ignored (cf. LEDERBERG and LEDERBERG 1956).

6. CONCORDANCE WITH STOCKER (1956b)

6.1 No irreconcilable differences obtain between the two studies, which differ primarily in the strains used, and in the emphasis given to the interpretation of the linearity of *E.*

6.2 In his material, STOCKER found a greater discontinuity in the distribution of lines per initial than I show in table 1, and his is flatter for the lower yields. We are in full agreement on the disproportionate partitions of figure 3. He has found six pedigrees (for my one, 2.7) in which an *E* cell was recovered late in the clone. His pedigree analysis of the linearity of *E* is more detailed, but it is impossible for it to

be complete 2.11 and the main evidence for this comes from the partition data. I have already commented on possible rare exceptions to *E*-linearity 5.3. These might be misclassifications due to the overlap of *E* and non-*E* distributions, or experimental errors, rather than valid exceptions. On the other hand they are sufficiently rare that STOCKER's data and mine cannot be shown to be statistically heterogeneous. Finally, we have used slightly different materials and, perhaps, techniques.

6.3 It has not been possible to make a direct test of the assertion that only an *E*-cell will form a trail in NGA. We agree that the incidence of trail-forming and of multilinear initials is about the same. The progressive increase in yield of trails as the NGA is diluted 3.3 suggests that this agreement in "standard NGA" is fortuitous, and the stochastic factors (such as entrapment by agar fibrils) are decisive in trail formation. The occurrence of three or four (out of 186) clones with more than one trail, 3.8, argues against either the linearity of *E*, or the one to one correspondence of trails with *E* cells.

6.4 I have tried to show 5.5 that the exact linearity of *E* is not crucial for its material interpretations. STOCKER's preference for 5.6 B1, the residual exogenote, is entirely plausible as a working hypothesis, but the alternative versions cannot be discounted at present.

SUMMARY

1. A hereditary *line* is an unbranched chain of descent, based on recurrent unequal division. Two aspects of linear inheritance were observed in studies of transduction in Salmonella: the persistent linear transmission of motility itself, and the (approximately) linear transmission of the trait "E", *viz.* the generation of *many* motile *lines*.

2. Initial motile cells were isolated from transductions of motility to a nonmotile mutant, and the progeny from these initials studied microscopically and in soft agar plates. Four percent of the initial cells gave stable motile recombinants, analogous to the transformations of other traits previously studied. The remaining cells gave descents which contained one or more motile lines. Many of these lines were studied to verify that a motile cell would regularly divide to give one motile, one non-motile offspring.

3. The motile lines are considered to arise from a non-dividing phenotypic residue, perhaps a flagellum, from a previous "abortive transduction" of a motility gene.

4. Some of the clones from motile initials had many (ten to forty) motile lines. The partition of potential lines at cell division was regularly unequal. Thus it was concluded that *E* is also inherited in linear or nearly linear fashion.

5. A number of hypotheses for this behavior were considered, notably that either a sterile (non-replicating) chromosome fragment or an intermediate product (phenotypic residue) of its action is responsible. Methods of distinguishing these hypotheses are discussed, but none are presently available for this material.

6. Various aspects of transduction mechanics (including early segregation in transformed clones, range of fragment size, position effect) are reviewed. Conceptual parallels for linear inheritance in genetics, development and ageing, and the semantics of "particle" are speculatively discussed.

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A GENETIC CHANGE ASSOCIATED WITH THE R LOCUS IN MAIZE WHICH IS DIRECTED AND POTENTIALLY REVERSIBLE*

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INTEGRITY of the gene in heterozygotes, first inferred from MENDEL's (1865) Pisum data and repeatedly confirmed since in a wide variety of organisms, is regarded as a universal principle in Mendelian heredity. Departures from the law have been reported from time to time, only to prove unfounded, in most instances at least, on close analysis. The present study is concerned with a seeming exception to the rule at the well-known *R* locus in maize conditioning aleurone and plant color.

All the R^r ♂ gametes (self-colored aleurone) produced by R^rR^{st} plants (heterozygous stippled aleurone) were found to give a different phenotype in testcrosses on r^rr^r ♀ ♀ (colorless aleurone) than the R^r ♂ gametes formed by R^rR^r sibs. The altered form of R^r in the R^rr^r testcross progeny is transmitted through the succeeding sporophyte generation and, therefore, is heritable.

The change in R^r is potentially reversible with equal regularity. R^r gametes formed by all R^rR^r plants derived from R^rR^{st} heterozygotes by selfing were found to have regained the standard (or near-standard) level of pigment-producing action in testcrosses with r^rr^r ♀ ♀.

The present communication is a preliminary account of this unusual phenomenon.

MATERIALS AND METHODS

The R^r gene used conditions self-colored (purple or red) aleurone when homozygous. The superscript in the symbol (EMERSON, ET AL. 1935) denotes that the allele also produces anthocyanin pigment in vegetative tissues of the plant. The other allele is R^{st} (stippled), which gives an irregular pattern of mostly small colored spots with sharply defined boundaries in the aleurone, the outer layer of endosperm cells.

Aleurone color alone could be scored in the present experiment (see later). The plant color action of the R^r and R^{st} genes was utilized only to identify R^rR^r, R^rR^{st}, and $R^{st}R^{st}$ plants in segregating families at the seedling and flowering stages. R^rR^r plants have red coleoptiles and anthers, whereas these tissues are green in $R^{st}R^{st}$ individuals. The anthers in the heterozygote, R^rR^{st}, are light red, although there is overlap with the R^rR^r class.

In order to improve readability of the text the plant color superscripts will be omitted from the gene symbols except as the complete designations are necessary to make the meaning clear. *R*, therefore, will be substituted for R^r, and r^r will be represented by *r*.

The *R* gene in question has been in the writer's cultures for ten years or more, without having disclosed any unusual properties. It is evident from STADLER's (1951)

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extensive work on this locus, however, that many *R* alleles of this class are only moderately stable as compared, for example, with most of the commonly occurring representatives of the A_1 and *C* loci, also concerned with anthocyanin pigmentation in maize. The particular *R* gene used is probably typical in this respect. Adequate tests of stability have not yet been made, but examination of about 14,000 kernels from a population of *RR* plants of this stock grown in isolation for another purpose revealed seven mottled kernels, indicative of mutations to or toward *r*, transmitted through the macrospores. These are not definitive data on mutation rate but they demonstrate, at least, that this *R* allele is not in the highly mutable category.

The R^{st} gene, on the other hand, is relatively unstable, mutating to self-color as frequently, perhaps, as 1 per 100 kernels in the present stock. Here again adequate data are not at hand. The figure given is based on kernel phenotype in the few stippled ears currently available to the writer.

Most R^r alleles, including the one in question, give an irregularly broken pattern of aleurone color when present with two *r* (colorless) genes in the triploid endosperm nuclei. This well-known phenomenon is termed "R-mottling". The R-mottling in the stock here used is much lighter in *RrrCcc* than in *RrrCCC* endosperms. That is to say, the dosage of *C* affects R-mottling, a fact of obvious importance in scoring kernels for intensity of pigmentation which seems not to have been recorded in the literature. The dosage effects of *R* alleles in general on aleurone color have not been studied in detail, but it is apparent that the action of the *R* gene here used is not simply additive in heterozygotes with *r*; *RRr*(*Ccc*) is much more than twice as heavily pigmented as *Rrr*(*Ccc*). Seemingly, therefore, each of the *R* genes in an *RRr*(*Ccc*) aleurone nucleus augments the pigment-producing action of the other. In view of the statement above concerning the modifying action of *C* on R-mottling, however, this non-linear effect may not be readily discernible in plants segregating both *R* and *C*.

The *R* and R^{st} stocks employed were modified forms of a relatively vigorous, fertile, and uniform dent inbred line, known commercially as W22. The initial outcross of W22 (A_1cr) to the A_1CR source was followed by five backcrosses to W22, before isolating the homozygous self-colored genotype, A_1CR, by selfing. The R^{st} gene then was substituted for *R* in a parallel sub-line. Three backcrosses to the recurrent, near-homozygous, self-colored parent were made in this case. The genetic backgrounds of the *R* and R^{st} strains, therefore, were both closely comparable and relatively homozygous.

A simple mating plan was followed. A single RR^{st} plant (Bk 701–1) in the third backcross generation of RR^{st} to W22 A_1CR was self-pollinated. The resulting 50% of self-colored kernels were planted under the family number 45–5, and consisted of the genotypes *RR* (endosperm, *RRR*) and RR^{st} (endosperm, RRR^{st}). The mottled kernels were given the pedigree number 45–6, and comprised the genotypes RR^{st} (endosperm, $RR^{st}R^{st}$) and $R^{st}R^{st}$ (endosperm, $R^{st}R^{st}R^{st}$).

Self-pollinated ears from $R^{st}R^{st}$, RR^{st}, and *RR* plants in the converted W22 inbred stock are illustrated in figure 1.

Several plants in each of the three classes of offspring (identified by anther color) were selfed, and pollen of each individual also was applied in turn to three A_1cr

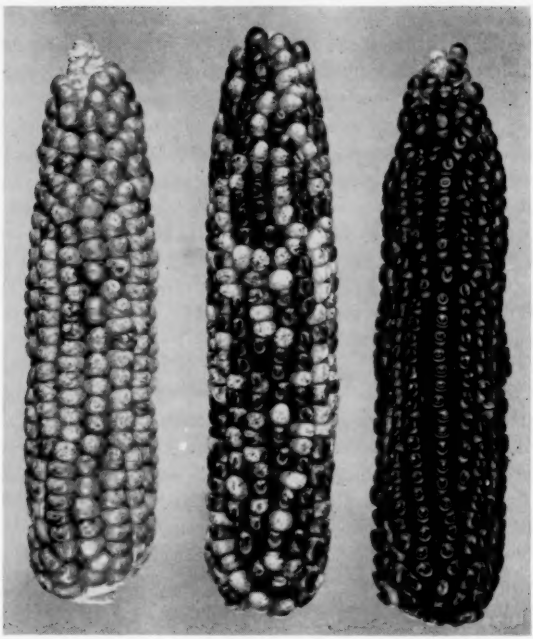

FIGURE 1.—Self-pollinated ears of homozygous self-colored (right), homozygous stippled (left), and the F_1 R^rR^{st} heterozygote (middle) in the W22 stock.

inbred lines. A few additional matings were made by placing pollen from *RR* and RR^{st} plants on $R^{st}R^{st}$ sibs.

Classification of *RR* and RR^{st}, and $R^{st}R^{st}$ plants, inferred in the field from anther color, was confirmed from the selfed ears obtained in the two families, 45-5 and 45-6, also used as the pollen source in the testcrosses on *rr*.

Three unrelated A_1Cr inbred strains of dent corn, W8, W22, and 4Co63, were used as pistillate parents in the testcrosses with the W22 *RR*, RR^{st}, and $R^{st}R^{st}$ sibs. Comparable results were obtained in all three sets of matings. Detailed analysis was limited, however, to the 4Co63 ears because they gave the fullest complement of seed and the sharpest differentiation of aleurone color. The 4Co63 stock was highly inbred, and was supplied originally by DR. G. F. SPRAGUE, Iowa State College, Ames.

The use of r^rr^r plants, which have colorless kernels but which form anthocyanin pigment in various vegetative tissues, as pistillate parents in the testcrosses limited observations in this experiment to effects on aleurone phenotype. Another series of testcrosses, using r^gr^g (colorless aleurone, green plant), rather than r^rr^r individuals as females, is necessary to determine whether the differential effect of R^r alleles emerging from R^rR^r and R^rR^{st} sibs on aleurone color, which is the subject of this report, extends to plant pigmentation also.

Samples of kernels from a few of the *rr* ♀ × *RR* ♂, *rr* ♀ × *RR*st ♂, and *rr* ♀ × *R*st*R*st ♂ testcross ears then were scored for amount of aleurone pigmentation. A dissecting microscope was used for this purpose which was fitted with a 20 × 20 reticule covering about 12 mm^2 at 27× magnification. The kernels were placed on the stage so that the silk scar occupied a given position just outside the edge of the reticule scale. Each of the 400 squares into which the field was thus divided then was scored as "+" if it contained any pigmented aleurone cells and "−" if it was entirely colorless. The sum of the positive scores, divided by 4, was taken as the color index for a kernel. Occasionally the control point on the kernel was shifted slightly to complete the field, and size of field varied somewhat depending upon curvature of the kernel surface. Neither of these factors, however, is a source of bias in the results. This scoring method is tedious, but it provides definitive quantitative data on the question at issue.

Progeny tests were necessary to identify the two overlapping classes of kernels resulting from the *rr* × *RR*st matings. A few kernels from the testcross ears were chosen whose color indexes approximated the two respective modal values. The plants grown from these kernels were either selfed or pollinated with inbred 4Co63, and pollen from them was applied to the 4Co63 stock (*A*$_1$*cr*). Parallel control matings were based on random kernels from the *rr* × *RR* and *rr* × *R*st*R*st testcross ears, respectively.

EXPERIMENTAL RESULTS

Distribution of the kernels in terms of color index from the testcrosses on 4Co63 *A*$_1$*cr* females of the *RR*, *RR*st, and *R*st*R*st sibs in families 45-5 and 45-6 are shown in table 1. The numerical results obtained from ears of the three classes are presented graphically also in figure 2.

The photographs of the testcross ears in figures 3 and 4 show that the phenotypic differences under consideration are conspicuous, even to the unaided eye. The respective kernel phenotypes are illustrated at a higher magnification in figure 5.

The rr ♀ × RR ♂ testcross

All the kernels from the two testcross ears of this type scored in detail gave high color indexes. The modal value was 98 in each case, that is, it lay at the upper limit of the scale. The remaining kernels were clustered in the adjacent classes. The testcross ears involving two other *RR* plants in family 45-5, examined by inspection only, showed comparable pigmentation.

These kernels, as expected for the *Rrr* endosperm genotype, were R-mottled, not self-colored. What the high scores for color index mean, therefore, is that most of the 400 units defined by the 20 × 20 reticule, sampling about 12 mm^2 in a given position on each kernel, contained one or more pigmented aleurone cells.

The rr ♀ × Rst*R*st *♂ testcross*

The homozygous stippled segregates from the selfed *RR*st plant (Bk 701-1) are represented in table 1 by two testcross families (lines 3 and 4). The modal color

TABLE 1

Distribution of kernels in terms of color index following the pollination of $r^r r^r$ ♀♀ *by* $R^r R^r$, $R^{st} R^{st}$, and $R^r R^{st}$ *sibs*

Line	Testcross	Aleurone color index class values																			
		3	8	13	18	23	28	33	38	43	48	53	58	63	68	73	78	83	88	93	98
	$r^r r^r$ ♀ × $R^r R^r$ ♂																				
1	6H97-1 × 45-5-13															2	4	6	11	15	27
2	6H97-4 × 45-5-32																	3	4	19	36
	$r^r r^r$ ♀ × $R^{st} R^{st}$ ♂																				
3	6H97-17 × 45-6-102			2	26	48	23	3	2	1											
4	6H97-16 × 45-6-103			3	19	32	23	11	2												
	$r^r r^r$ ♀ × $R^r R^{st}$ ♂																				
5	6H97-8 × 45-5-104	58	5	4	10	24	12	13	4	1											
6	6H97-10 × 45-6-1	46	10	8	22	23	10		2	1	2	1	1			1			1		1
7	6H97-11 × 45-6-4	44	5	5	3	11	12	7	6	1	1								1		

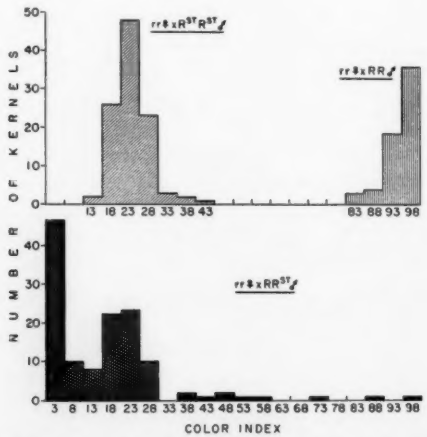

FIGURE 2.—Distributions of kernels, according to color grade, from pollination of $r^r r^r$(*c c*) by sibs from $R^r R^{st}$, selfed. $R^r R^r$ ♂ (upper right); $R^{st} R^{st}$ ♂ (upper left); and $R^r R^{st}$ ♂ (below).

index for both these families is 23, with the other entries distributed closely around this value.

Occasional self-colored kernels resulted from the *rr* × $R^{st}R^{st}$ matings. None happened to occur in the areas sampled on the two ears represented in table 1. These uniformly colored kernels probably represent mutations of R^{st} to an *R* allele with high expressivity for aleurone color in the *Rrr* combination.

The rr ♀ × RRst testcross

It would be expected, on the basis of the data from the testcrosses involving *RR* and $R^{st}R^{st}$ plants presented above, that two discrete classes of kernels would be formed following the *rr* ♀ × *RR*st ♂ mating, one with a modal value of 23 and the other with a modal value of 98. A different result, however, was found.

Nine testcross ears on the 4Co63 A_1cr inbred line were obtained, representing the same number of *RR*st sibs used as the pollen parents. The distributions on all the ears were alike, and random samples of the kernels on three ears were scored for pigmentation under a binocular microscope. The results are entered in lines 5–7 in table 1. Plant 45-5-104, having been grown from a self-colored kernel, will have received the R^{st} allele through the pollen and *R* through the female gametophyte. The converse is true for the other two male parents, 45-6-1 and 45-6-4.

The data show that well-defined, but overlapping, bimodal distributions resulted from each of the *rr* ♀ × *RR*st ♂ testcrosses and that one mode only conforms to expectation based on the data from the corresponding homozygotes. This mode, at or near 23, matches that for the *rr* × $R^{st}R^{st}$ testcross.

The other mode is at 3, a value which was not found on similarly testing the two respective homozygous genotypes. If these kernels are of the *Rrr* class then the pigment-producing potential of the *R* male gametes involved in their origin has been so reduced that the color index of the *Rrr* endosperm progeny has been shifted from the top of the scale to a position near the bottom. It is significant, of course, that the entire group of kernels is so affected.

The data in lines 6 and 7 in table 1 show that the range in color index following the *rr* ♀ × *RR*st ♂ matings may extend upward on the scale beyond the limits for the $R^{st}R^{st}$ testcross. The relation of these few kernels to the others in the populations is unknown at present.

Testcrosses on homozygous stippled

Pollen from three *RR* and three *RR*st plants in families 45-5 and 45-6 was applied also to $R^{st}R^{st}$ sibs. Inspection of the resulting ears showed that the $RR^{st}R^{st}$ kernels from the $R^{st}R^{st}$ ♀ × *RR* ♂ matings were distinctly darker in aleurone color than the corresponding class of kernels from the $R^{st}R^{st}$ ♀ × *RR*st ♂ crosses. This finding demonstrates that the difference in pigment-producing capacity of *R* male gametes produced by the *RR* and *RR*st sibs is not restricted in its expression solely to the *Rrr* genotype employed in the main group of testcrosses; the difference is shown also in endosperm cells whose nuclei contain the R^{st} allele. Furthermore, it is apparent that the *R* gene derived from an *RR* male parent is not detectably altered in pigment-producing action in an $RR^{st}R^{st}$ endosperm.

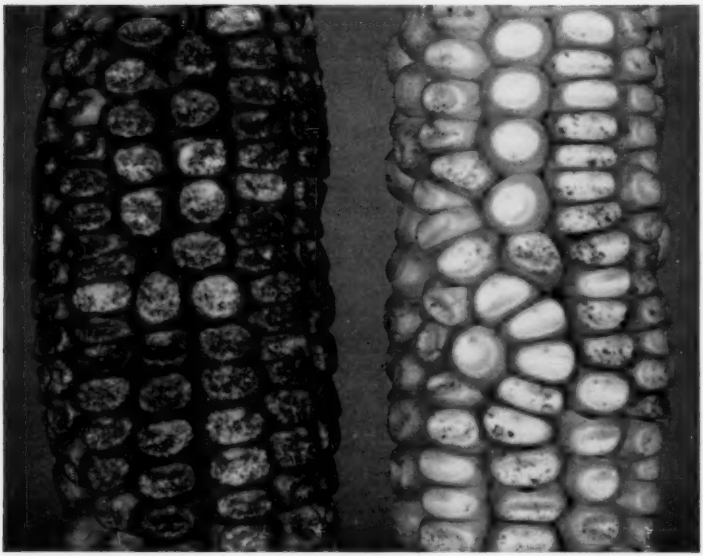

FIGURE 3.—Testcross ears of $r^r r^r$ ♀ × $R^r R^r$ ♂ (left) and $r^r r^r$ ♀ × $R^r R^{st}$ ♂ (right). The kernels on the left hand ear show the standard level of R-mottling in $R^r r^r r^r$(*C c c*) endosperms from 4Co63 ♀ × W22 A_1C R^r crosses. The pollen parents were sibs from $R^r R^{st}$ selfed.

It has been mentioned already that the unrelated inbred lines W8 and W22 when used as pistillate parents in the testcrosses yielded the same kind of result as the 4Co63 stock. These three strains well may carry somewhat different forms of the *r* allele. Certainly they differ markedly in their residual genotypes.

Kernel phenotypes obtained on selfing RR^{st} plants

It would have been desirable to have obtained *RR* ♀ × *rr* ♂ and RR^{st} ♀ × *rr* ♂ testcross ears also. These reciprocal matings were not made. The probable results, however, may be inferred from the selfed ears on the *RR* and RR^{st} plants used as staminate parents in the testcrosses with the 4Co63 A_1Cr line. All the kernels on the selfed *RR* plants in family 45-5 were uniformly self-colored, and were indistinguishable in phenotype from those in the W22 A_1CR stock culture from which the *R* allele originally was derived. The right-most ear in figure 1 is representative.

This means that the *R* allele extracted in homozygous condition from an RR^{st} parent gives fully colored aleurone, a result in agreement with the *rr* ♀ × *RR* ♂ testcross data, but which raises the questions of how and when the modified *R* allele emerging from the hybrid is restored to the standard level of pigment-producing action.

The self-pollinated ears on the RR^{st} sibs in families 45-5 and 45-6 bore approxi-

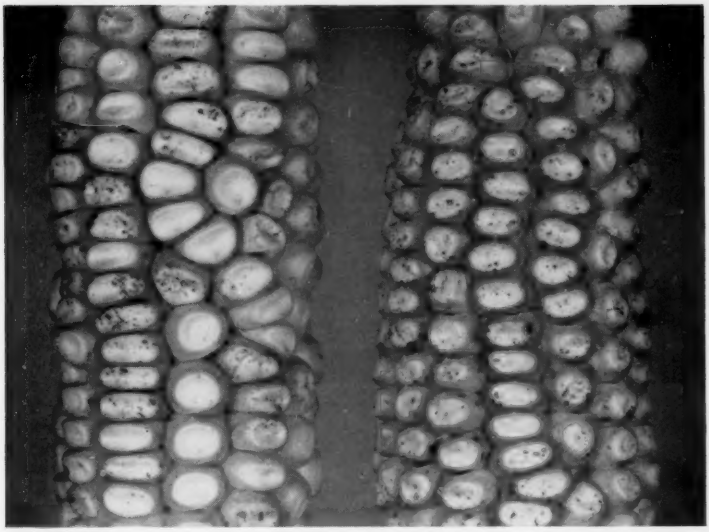

FIGURE 4.—Testcross ears of $r^r r^r$ ♀ × $R^{st}R^{st}$ ♂ (right) and $r^r r^r$ ♀ × $R^r R^{st}$ ♂ (left). Half the kernels on the latter ear are stippled, like those on the right hand ear; the other half of the kernels show the weakly pigmented phenotype characteristic of modified R (R') in $R'r^r r^r$(C c c) endosperms. The pollen parents were sibs from $R^r R^{st}$, selfed. The ear at the left is the same one shown at the right in figure 3.

mately equal numbers of self-colored and stippled kernels. This is a distribution with which maize geneticists are familiar and which has been interpreted on the conventional grounds that R is recessive in the endosperm to two doses of R^{st} and, conversely, R^{st} is recessive to two doses of R. The self-colored kernels on these ears were as darkly pigmented as those in the W22 A_1CR stock culture. This is not the result expected if the R gametes from the RR^{st} heterozygotes were reduced in color-determining potential to the level revealed in the testcrosses of the same plants with 4Co63 A_1cr and had remained at this level in the RRR and RRR^{st} aleurone cells. The implication is that the modified R allele entering these two classes of endosperm cells regains the standard level of color-producing action.

The progeny from the testcross kernels

F_1 kernels from two 4Co63 rr ♀ × RR^{st} ♂ testcross ears (6H97 × 45-6-1, and × 45-6-2) were separated according to aleurone pigmentation into two groups provisionally designated "light" and "stippled". These kernels then were entered in a progeny test together with seeds from two ears each from the other two test matings, rr ♀ × RR ♂ and rr ♀ × $R^{st}R^{st}$ ♂. The resulting plants either were selfed or were pollinated with the 4Co63 rr stock; also pollen from each of them was applied to 4Co63 rr ear shoots.

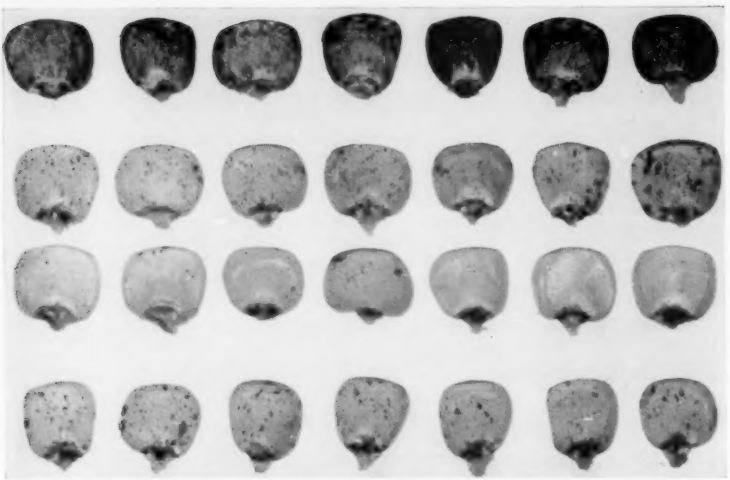

FIGURE 5.—Representative testcross kernels obtained on pollinating $r^r r^r (c\ c)$ with $R^r R^r$ (top row), $R^{st} R^{st}$ (bottom row) and $R^r R^{st}$ (two middle rows) sibs from $R^r R^{st}$, selfed. The kernels in the middle rows represent partially overlapping classes, stippled (above) and mostly, or all, modified R (R') (below).

(1) Dark mottled (*Rr*) F_1 kernels from the *rr* ♀ × *RR* ♂ testcross ears

Matings were completed with 12 *Rr* plants of this origin. The four *Rr*(*Cc*) ♀ × *rr*(*cc*) ♂ crosses which were made yielded ears carrying about 25% normally self-colored kernels. The other eight F_1 *Rr* plants were selfed, and gave approximately two normally self-colored to one normally R-mottled kernels in the colored class (see fig. 6, left). The colored kernels on the 12 ears obtained on applying pollen of these individuals to the 4Co63 *rr* inbred line were typically dark mottled.

Two conclusions may be drawn from these results: (a) the standard level of pigment-producing action inherent in *R* gametes formed by *RR* plants extracted from RR^{st} individuals, selfed, as shown in the endosperm of the original *rr* ♀ × *RR* ♂ testcross, is maintained in, and transmitted by, *Rr* sporophytes, and (b) the *r* gene from the 4Co63 stock does not alter the aleurone pigment-producing action of *R* in *Rr* heterozygotes.

(2) Stippled F_1 kernels ($R^{st}r$) from the *rr* ♀ × $R^{st}R^{st}$ ♂ testcross ears

Nine F_1 plants of this class were tested. The results were entirely regular. $R^{st}r$ individuals, either selfed or pollinated by 4Co63 *rr*, gave typically stippled kernels in the expected Mendelian proportions. Likewise, when pollen from the $R^{st}r$ individuals was placed on 4Co63 *rr* ear shoots the colored kernels resulting showed the grade of stippling which is usual for the $R^{st}rr$ genotype.

(3) "Stippled" F_1 kernels from the *rr* ♀ × RR^{st} ♂ testcross ears

Ear progenies were obtained from six plants grown from this class of kernels. Four individuals were selfed and two were pollinated with pollen from the 4Co63

line. The colored kernels resulting from both these kinds of matings were typically stippled. Furthermore, the seeds obtained on applying pollen from the six F_1 plants to 4Co63 *rr* ear shoots showed the same stippled phenotype as obtained in the corresponding crosses referred to in the preceding paragraph. Evidently, therefore, the 50% of kernels on the ears from the original 4Co63 *rr* ♀ × R^rR^{st} testcrosses of the "stippled" phenotype were genotypically stippled. Furthermore, it may be concluded that the R^{st} allele is not regularly altered in pigment-producing action in heterozygotes either with *R* or *r*.

Two exceptional plants also were found in this group which may have resulted from misclassification due to overlap of the "stippled" and "light" kernel phenotypes on the parent ear. One plant, on selfing, and the other, on being pollinated with 4Co63 *rr*, yielded self-colored, rather than stippled, kernels. The colored kernels resulting from application of their pollen to the 4Co63 *rr* stock, likewise, were not stippled but were similar in phenotype to the corresponding class in the next following group of matings.

(4) "Light" F_1 kernels from the *rr* ♀ × RR^{st} ♂ testcross ears

Twelve plants grown from this exceptional class of weakly pigmented kernels, in addition to the two individuals mentioned just above, were tested. Nine of the plants were selfed and three were pollinated by 4Co63 *rr*. All the resulting ears but two bore typical self-colored kernels in the proportions expected if the parent plants were *Rr*

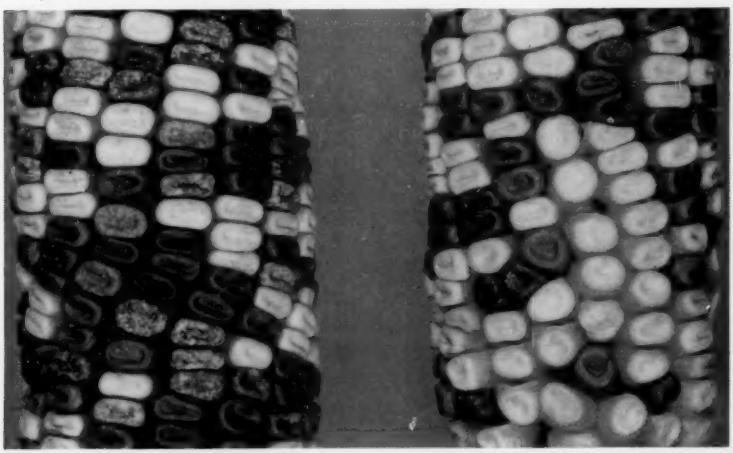

FIGURE 6.—These ears illustrate the transmission of R^r and R' in heterozygotes with r^r. The ear on the left was borne by a self-pollinated R^rr^r *C c* plant resulting from a cross between 4Co63 r^rr^r *c c* and an R^rR^r *C C* individual derived from R^rR^{st} by selfing. It carries, therefore, a "restored" R^r. The ear shows a typical Mendelian distribution of kernels: 6 self-colored ($R^rR^rR^r$ and $R^rR^rr^r$ endosperms), 3 mottles ($R^rr^rr^r$ endosperms), and 7 colorless. The ear on the right carries R' instead of R^r. It was produced by self-pollinating a plant grown from a weakly pigmented kernel derived from a cross between 4Co53 r^rr^r *c c* and an R^rR^{st} *C C* individual originating from R^rR^{st} *C C* by selfing. Note the few faintly mottled kernels ($R'r^rr^r$ endosperms) and the apparent overlap of the genetically mottled and colorless classes. The proportion of self-colored kernels is 6/16 on both ears.

and self-color was associated with the *RRR* and *RRr* endosperm genotypes. The other two ears segregated 9 light mottled and 7 colorless, the mottling pattern being somewhat like, but not identical with, that of stippled. The relation of these two ears to the others in the group is not known at present. It is suspected, however, that they represent a mutant form of stippled which is under study in a related stock.

The mottled kernels, (presumably *Rrr*) on all seven selfed ears segregating self-color, were much less extensively pigmented than the corresponding seeds in group (1), above (see fig. 6, right). Furthermore, the proportion of mottled kernels varied from ear to ear, falling below the level expected on Mendelian grounds. This suggests that frequently the mottling was subliminal.

Of prime significance also is the fact that the colored kernels resulting from the pollination of 4Co63 *rr* by all 12 of these plants were weakly pigmented throughout. These individuals stand in sharp distinction, therefore, to those in group (1), above, in which the *R* allele transmitted gave typical dark R-mottling in the *Rrr* endosperm progeny. Evidently the "modified *R*" allele present in the "light" testcross kernels is not restored to the normal level of pigment-producing action in sporophytes heterozygous for the 4Co63 *r* gene.

Further study is necessary before the "light" class of testcross kernels can be

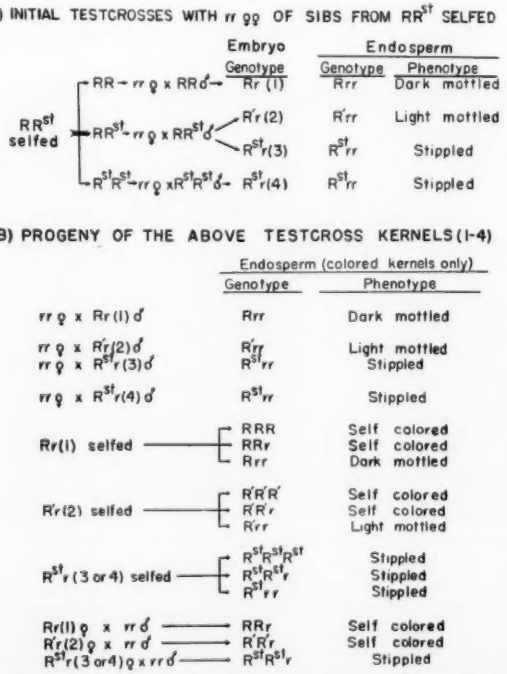
(A) INITIAL TESTCROSSES WITH rr ♀♀ OF SIBS FROM RR^st SELFED
Embryo Genotype
Endosperm Genotype
Endosperm Phenotype
RR selfed
RR → rr ♀ x RR♂ → Rr (1) Rrr Dark mottled
RR^st → rr ♀ x RR^st♂ → R'r (2) R'rr Light mottled
R^st r (3) R^st rr Stippled
R^st R^st → rr ♀ x R^st R^st ♂ → R^st r (4) R^st rr Stippled
(B) PROGENY OF THE ABOVE TESTCROSS KERNELS (1-4)
Endosperm (colored kernels only)
Genotype
Phenotype
rr ♀ x Rr (1) ♂ Rrr Dark mottled
rr ♀ x R'r (2) ♂ R'rr Light mottled
rr ♀ x R^st r (3) ♂ R^st rr Stippled
rr ♀ x R^st r (4) ♂ R^st rr Stippled
Rr (1) selfed
RRR Self colored
RRr Self colored
Rrr Dark mottled
R'r (2) selfed
R'R'R' Self colored
R'R'r Self colored
R'rr Light mottled
R^st r (3 or 4) selfed
R^st R^st R^st Stippled
R^st R^st r Stippled
R^st rr Stippled
Rr (1) ♀ x rr ♂ → RRr Self colored
R'r (2) ♀ x rr ♂ → R'R'r Self colored
R^st r (3 or 4) ♀ x rr ♂ → R^st R^st r Stippled

FIGURE 7.—Outline of the mating plan, and summary of the breeding results.

characterized adequately. Meanwhile, the following inferences concerning them may be drawn from the facts at hand. (a) The very lightly pigmented kernels on the *rr* ♀ × *RR*st ♂ testcross ears evidently owe their origin to fertilization by counterparts of standard *R* male gametes. The two observations which support this conclusion are, first, the alternative class of kernels expected from this mating (stippled) is otherwise accounted for, and, secondly, with two possible exceptions, self-colored kernels of standard phenotype are formed when these plants are selfed or pollinated with *rr*. (b) Modified *R* is not restored to the standard level of pigment-producing action in sporophytes carrying the 4Co63 *r* allele. That is to say, *R* male gametes arising from *RR*st plants, regularly are genetically different from standard *R* gametes. (c) The variable degree of mottling attributable to modified *R* observed in the above testcrosses may be due to the action of modifying factors segregating in these F_1 male parents. Or, perhaps, modified *R* is a composite class, or it may be subject to frequent secondary changes.

A summary of the breeding data obtained is given in figure 7.

DISCUSSION

The evidence shows that *R* male gametes arising from *RR* and *RR*st sibs in an inbred maize line regularly differ in capacity to induce aleurone pigmentation in matings with *rr* pistillate plants. The difference is not limited to a phenotypic effect in the immediately resulting endosperm but may be carried through the sporophyte and then expressed in the following endosperm generation. If the modified *R* allele formed by *RR*st heterozygotes is designated *R'* then the respective *Rr* and *R'r* offspring of *rr* ♀ × *RR* ♂ and *rr* ♀ × *RR*st ♂ matings regularly differ from each other in breeding behavior: the colored kernels from *rr* ♀ × *Rr* ♂ testcrosses show the standard dark R-mottling, whereas those from *rr* ♀ × *R'r* ♂ testcrosses are weakly pigmented. Thus the change from *R* to *R'* persists from the one sporophytic generation to the next, and hence may properly be termed genetic.

The *R*st male gametes formed by *RR*st plants, on the other hand, are indistinguishable in their effect on aleurone pigmentation from those formed by sibs homozygous for stippled. The *R*st allele, therefore, is not regularly altered in heterozygotes with *R*. It is to be borne in mind, however, that rare or inconspicuous changes in *R*st would not have been revealed by the tests made.

Under certain conditions *R'* reverts to *R* (or, strictly speaking, to a high level of pigment-producing activity) with a regularity equal to that of the original change of *R* to *R'*. This was demonstrated in the case of the *RR* offspring of self-pollinated *RR*st plants. The *R* male gametes from these extracted homozygotes, when used in testcrosses with *rr* ♀♀, were found to give darkly mottled kernels. Furthermore, this high level of action was shown to be retained in the *Rr* sporophytic offspring from *rr* ♀ × extracted *RR* ♂ matings. Evidently *R'* regularly changes back to *R*, or a similar allele, at one or another stage of development in *RR* plants derived from *RR*st selfed individuals. Additional tests are necessary to determine the closeness of correspondence in phenotypic effect between reverted *R* and the original *R* and the relative stabilities of these factors.

It is unlikely that the change in aleurone phenotype from *R* to *R'* results from

genetic alteration of a cytoplasmic component in RR^{st} plants. One might suppose that under the combined action of the R and R^{st} alleles in heterozygotes a substance is formed outside the cell nucleus which interferes with a particular reaction involved in anthocyanin formation and that, as a result, R action is partially suppressed in the offspring of the heterozygotes. There are three principal reasons why such an explanation is improbable. (1) It would be necessary to impute to the substance the capacity to interfere with anthocyanin development at extraordinarily high dilutions, because the alteration is carried in $R'r$ plants through the long cell lineage represented by a sphorophytic generation. (2) If this were the mechanism of aleurone pigment reduction one would not expect the RRR and RRR^{st} endosperms formed on self-pollinating RR^{st} plants to be self-colored, because they would have received a large amount of modified cytoplasm through the female gametophyte. They are, in fact, fully pigmented. (3) If the assumed interaction of R and R^{st} leading to production of the cytoplasmic substance in question is fully effective before about the 15th day of endosperm development, when the single layer of aleurone cells is formed, $RR^{st}R^{st}$ endosperms resulting from $R^{st}R^{st}$ ♀ × RR ♂ matings should be no more heavily pigmented than $RR^{st}R^{st}$ endosperms arising from the $R^{st}R^{st}$ ♀ × RR^{st} ♂ mating. Actually they are much darker in color.

Another type of explanation in cytoplasmic terms can be formulated on the supposition that a self-propagating extranuclear cell component, a plasmid in LEDERBERG'S (1952) useful terminology, concerned with anthocyanin formation is genetically changed in RR^{st} plants in response to the combined action of the R and R^{st} alleles. The same results would be expected if $R^{st}R^{st}$ plants regularly carry such a plasmid. A phenomenon of this sort involving the iojap (*ij*) gene is known in maize (RHOADES 1943). The plastid mutations which RHOADES observed in the presence of the *ij* gene, however, were transmitted by the egg but not through the pollen. In fact, there is no evidence in Zea of pollen transmission of any extranuclear genetic component even among the different forms of cytoplasmically determined, but gene modified, male sterility which corn breeders are now exploiting on a major scale.

A hypothesis based on the assumption that in cells carrying RR^{st} nuclei a plasmid affecting anthocyanin development undergoes mutation encounters all the difficulties, except persistence, met with in postulating a passive substance having the same effect. Such an explanation is rendered unlikely by the breeding results obtained with Rr and $R'r$ plants arising from pollination of rr ♀ ♀ by RR and RR^{st} sibs from selfed RR^{st} individuals. It will be recalled that the colored kernels formed following the rr ♀ × Rr ♂ matings showed the level of pigmentation normal for Rrr endosperms, whereas the rr ♀ × $R'r$ ♂ testcrosses gave all weakly pigmented kernels in the colored class. The colored kernels from both these matings should have shown reduced pigmentation if a self-propagating cytoplasmic element of mutant type had arisen in the ancestral RR^{st} plant. It appears improbable on the basis of the existing evidence, therefore, that the persistent change in R expression arising in RR^{st} plants has a cytoplasmic basis.

Further exploration of the possibility that a plasmid of a novel type is involved, however, is indicated. It would be necessary to assume that such a cytoplasmic entity regularly is transmitted through the pollen and also that it survives differ-

entially according to the genotype at the *R* locus. Discussion of an explanation based upon these considerations will be deferred until the relevant tests have been made.

Tentatively, it may be concluded that the basis of the difference between the *R* and *R′* expressions is intrachromosomal. The transmission data implicate the *R* locus. Families 45-5 and 45-6 were closely inbred and were homozygous for the known dominant genes conditioning aleurone pigmentation, except at the *R* locus. Even though the plants used as pistillate parents in most of the testcrosses were *cc rr*, rather than *CC rr*, the *RR* and RR^{st} individuals from RR^{st}, selfed, evidenced the phenomenon in question, whereas the $R^{st}R^{st}$ sibs did not show it. Furthermore, all the $R^{st}r$ progeny from this testcross again were regular in breeding behavior in a further testcross on *cc rr* ♀ ♀. In addition, the *R′* male gametes from the RR^{st} segregates in families 45-5 and 45-6 gave a different endosperm phenotype than those from *RR* sibs in the testcrosses with *AA CC* $R^{st}R^{st}$ ♀ ♀. It is clear, therefore, that it is the *R*, not the *C*, locus which is directly involved.

The present data do not serve, however, to distinguish between a change at the *R* locus itself and one at a nearby site in the same chromosome. It is evident, nevertheless, that the alteration in pigment-producing action occurring in RR^{st} heterozygotes can not be explained in terms of transfer by conventional crossing over of some factor from the R^{st} to the *R* chromosome.

For purposes of experimental analysis, at least, it is convenient to recognize two facets to the general problem. The first is concerned with "induction" of the change of *R* to *R′* in RR^{st} plants and the second with the "restoration" of normal (or near-normal) pigment-producing action of *R* in the *RR* offspring of the heterozygotes. Whether these phenomena are the two sides of the same coin, so to speak, is not yet evident.

Little can be said at present regarding the conditions of induction, other than that the change occurs regularly in RR^{st} plants and does not occur when the same *R* allele is present in heterozygotes with the 4Co63 *r* allele. Whether R^{st} is unique in this respect among the numerous *R* alleles which are known is a question of interest, as is the range of *R* alleles which can be altered in heterozygotes. There is no evidence at present concerning the stage of development of RR^{st} plants at which the change from *R* to *R′* occurs.

A clue to a condition of reversion is afforded by the endosperm phenotypes which appear when RR^{st} and *R′r* plants are selfed and when the latter genotype is pollinated with 4Co63 *rr*. Half the kernels in selfed RR^{st} ears are self-colored. The embryos concerned comprise two classes, *RR* and RR^{st}, and the accompanying endosperms would be expected to be of the genotype *RRR* and RRR^{st} (family 45-5). The remaining 50% of kernels on these ears are stippled. The embryos in this class of seeds are $R^{st}R^{st}$ and RR^{st}, and the endosperms should be $R^{st}R^{st}R^{st}$ and $RR^{st}R^{st}$, respectively (family 45-6). Likewise, when *R′r*(*Cc*) plants are selfed about 9/16 of the kernels are self-colored, the proportion expected if the *R′R′R′* and *R′R′r* endosperm genotypes give full pigmentation. Furthermore, the 25% of colored kernels obtained on pollinating *R′r*(*Cc*) by *r*(*c*) all are self-colored.

The common denominator of these observations is that a cell whose nucleus contains two or three modified *R* alleles (*R′R′* in the sporophyte; *R′R′r* or *R′R′R′* in the endosperm) is potentially able to produce the self-colored phenotype. This evidence

suggests that in a nucleus containing two (or three) modified R's, R' regularly reverts to R. Credence is given this interpretation by the fact that the R allele in homozygous R sporophytes derived from RR^{st} plants by selfing gives the level of aleurone pigmentation characteristic of the standard R in crosses on rr ♀ ♀, whereas the corresponding class of gametes from $R'r$ individuals give weak pigmentation in the same test.

If this explanation is valid then sporophytes which are homozygous modified R ($R'R'$) are unobtainable. They change in all cases to RR.

It would be premature to conclude from these results that the presence of two R' alleles in the nucleus is a necessary condition of reversion to R. One would like to know, for example, whether restoration also occurs in R' plants heterozygous with the R allele present in the W22 A_1CR stock culture, which has been kept free of R^{st}, and with the occasional mutant r^r and R^g alleles to which this R allele presumably gives rise. It is possible that it is the specific property with respect to which R' differs from R that promotes the restoration of R' to R, but this has not been proven.

Genetic change at a locus in response to the presence of a particular allele in the homologous chromosome has been validated in a few other cases, some of them of long standing in genetics literature. These trans-allelic effects are so obscure, for the most part, however, that at present one can only conjecture what the several examples may have in common.

EMERSON (1929) reported that the unstable variegated pericarp allele (P^{vv}) in maize mutated to stable self-color (P^{rr}) about 2.8 times as frequently in heterozygotes as in homozygous variegateds. WOOD and BRINK (1956) recently have demonstrated that the difference in mutability of P^{vv} in the two classes of plants can be accounted for satisfactorily in terms of dosage action of Modulator (Mp), a transposable factor assumed to be a component of the P^{vv} allele. This is not an isolated case of its kind. The reddish-a character in *Drosophila virilis* (DEMEREC 1941), globifera in Antirrhinum (HERTWIG 1926), striping in Celosia (KIHARA 1932), Dotted (RHOADES 1936) and Activator (MCCLINTOCK 1951) in maize are parallel, in certain respects at least. Particularly noteworthy in this connection is MCCLINTOCK's (1951, 1956) demonstration of the widespread occurrence in maize of various intrachromosomal elements which she postulates as extragenic and as functioning in the control of gene action and mutation.

MITCHELL (1955a) recently has described the occurrence of wild type offspring in Neurospora following crosses between *pdxp* and *pdx*, two apparently allelic mutants affecting pyridoxine requirement, under conditions which made both ordinary back mutation and recombination at the locus by conventional crossing over unlikely explanations. The chromosome carrying *pdx* in $+/pdx$ heterozygotes also was observed to become wild type at this locus without recombination of closely linked markers. MITCHELL (1955b) suggests that the genetic changes of mutant to wild type in both these cases are mediated at meiosis by a direct interaction of some sort between the alleles. LINDEGREN (1953) previously had advanced an explanation of this type to account for the occurrence in certain yeast hybrids of asci containing one class of spores in excess of the number expected on regular Mendelian grounds.

Four niacin-requiring mutants at the q locus in *Neurospora crassa*, which are highly stable in vegetative tissue and in matings *intra se*, occasionally give rise to

wild type progeny when crossed with each other. ST. LAWRENCE (1956) has shown that the occurrence of niacin-independent offspring in these cases probably is not the result of recombination following crossing over at the *q* locus. She points out that, in the light of MCCLINTOCK's (1951) recent work, one possible explanation is that (1) the mutant phenotypes resulted from insertion at the *q* locus of chromosomal material which suppresses action of the wild type allele and (2) reversion to wild type involves removal from the locus of the inserted material. It is inferred that interactions occur between mutant alleles in heterozygotes which affect the frequency of the latter event.

It has been reported by NOUJDIN (1944) that in *Drosophila melanogaster* structural homozygosity and heterozygosity for the scute-8 inversion not only influence the mosaic expression of the closely linked genes yellow and achaete but also condition chromosome changes whereby the effects on mottling tend to persist irregularly in subsequent generations. The writer is not aware that this latter claim has been verified by others. The cytological findings of PROKOFYEVA-BELGOVSKAYA (1948), on similar material, however, lend support to NOUJDIN's contention. LEWIS (1950), commenting on a later report of NOUJDIN (1946) that the induced changes had disappeared in later generations, calls attention to the possibility that modifying genes may have contributed significantly to the observed effect.

The "barrage" phenomenon in the ascomycete, *Podospora anserina*, reported by RIZET (1952), resembles the maize case described here in that it involves a genetically controlled, heritable, change which is both regularly occurring and reversible. Although the facts presently available point to the cytoplasm as the seat of the alteration in Podospora, whereas the change in maize appears to be intrachromosomal, too little is known of the nature and expression of both changes to be certain that they are basically unlike.

Normally when two strains of Podospora make contact on solid medium in a petri dish the hyphae interpenetrate for a few millimeters, anastomose, and the mycelium becomes pigmented. Two strains are said to form a "barrage" when, on approaching each other, the hyphae branch and intertwine to form a dense mycelial network without anastomoses and without pigmentation of the hyphal tips. *S* and *s*, two strains differing in a single gene, regularly form barrages when confronted with each other. No *s* progeny, however, are obtained from $S \times s$ crosses. Instead a modified *s* type (s^s) appears in addition to *S*, which does not form barrages with either *S* or *s*. The s^s strain is highly stable vegetatively, and $s^s \times s^s$ crosses give s^s progeny. Spontaneous changes of s^s to *s* occur locally, which then tend to spread throughout the s^s mycelium. An s^s strain thus may be changed to *s* at will by putting it in contact with *s* mycelium. RIZET found that when *s* strains are fertilized by s^s microconidia the asci resulting contain only *s* spores. On the other hand, if the reciprocal cross is made most of the asci then contain only s^s spores. RIZET concludes that s^s strains carry an unchanged *s* gene, and that under the influence of the *S* gene in *Ss* heterozygotes a cytoplasmic change is induced premeiotically which conditions the s^s phenotype. EPHRUSSI (1953) commenting on RIZET's results states that "One can, of course, imagine that one of the mycelial types, either *s* or *S*, owes its character to the presence, the other to the absence of some sort of cytoplasmic particles, and that the two changes, from *s* to *modified s*, and the reverse, correspond, one to a 'con-

tamination', the other to a 'decontamination'. But many difficulties appear when one tries to account for all the known facts on the basis of such an hypothesis."

ESSER (1955) recently has reported results involving incompatibility alleles in Podospora which resemble those of RIZET. A directed change in phenotype regularly occurs, the genetic basis of which, however, is not clear from the limited data presented.

The nature of the difference in maize between what are here assumed to be chromosomal entities symbolized by R and R' is now largely speculative. The ready reversibility of the R to R' change suggests that the basic specificity for pigment production inherent in R is present in R' also, and that the distinction between the factors is extrinsic to this specificity. Whether the difference between R and R' actually is intrachromosomal, as here postulated, or rests upon an R-controlled plasmid which is pollen transmitted is a major issue. If it proves to be intrachromosomal then it becomes important to know whether the mechanism involved is integral with the R locus. Speculation concerning the phenomenon may well be deferred until the factual basis is established whereby more convincing answers to these questions can be given.

SUMMARY

The study is concerned with a genetic change in an R^r (self-colored aleurone color) allele in maize which regularly occurs in heterozygotes with R^{st} (stippled aleurone). The change is reversible with equal regularity under certain conditions.

Pollen from R^rR^r, $R^{st}R^{st}$, and R^rR^{st} sibs obtained by self-pollinating an R^rR^{st} plant in an inbred line was placed on r^rr^r individuals in another inbred line. The resulting kernels then were scored quantitatively for aleurone pigmentation. The observed modal color indexes were 98 from the R^rR^r homozygote and 23 from the $R^{st}R^{st}$ homozygote (scale 0–100). The r^rr^r ♀ × R^rR^{st} ♂ testcross gave a bimodal distribution with one mode at, or near, 23 and the other at 3. Progeny tests showed that the latter group of kernels (mode = 3) were the counterparts of the R^rr^r seeds obtained in the r^rr^r ♀ × R^rR^r ♂ testcross which gave a modal value for color of 98. Evidently the pigment-producing potential of the R^r male gametes formed by R^rR^{st} heterozygotes regularly is much lower than that of R^r male gametes from R^rR^r sibs. The determinative action of the R^{st} allele is not altered in R^rR^{st} plants. The altered form of R^r, termed R', emerging from R^rR^{st} heterozygotes was transmitted in $R'r^r$ plants through a sporophytic generation. The change, therefore, is genetic, and the breeding facts show that it is associated with the R locus. No evidence was obtained that an extranuclear genetic factor is involved.

R' regularly reverts to R^r, or a similar allele, in the homozygous offspring of selfed R^rR^{st} plants. Thus homozygous $R'R'$ appears to be unobtainable. Reverted R^r is similar to, although possibly not identical with, R^r in the inbred stock culture from which the allele was derived. Kernels with self-colored aleurone appear in the expected Mendelian proportion when R^rr^r and $R'r$ plants are selfed. This evidence suggests that in cells containing two or three R' alleles, but not one only, R' reverts to R^r.

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STUDIES ON DIFFERENT CLASSES OF MUTATIONS INDUCED BY RADIATION OF DROSOPHILA MELANOGASTER FEMALES[1]

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DELETERIOUS mutations are produced in the germ plasm when animals are exposed to total body radiation. The ovaries of most higher animals contain germ cells at different stages of development, and these cells may be expected to be affected to different degrees by ionizing radiation. It is, therefore, of both practical and theoretical interest to study the mutation frequency in successive batches of germ cells produced by females subsequent to radiation exposure, since it is important to know whether or not mutated cells are eliminated from the germ line and if mutant-free eggs are eventually produced.

OÖGENESIS

The process of oögenesis in the fruit fly, *Drosophila melanogaster*, has been described recently (KING, RUBINSON, and SMITH 1956). Each of the two ovaries of this insect is comprised of an average of 12 ovarioles, and these tubes are differentiated in turn into an anterior germarium and a series of egg chambers. The sixteen cells (15 nurse cells and the primary oöcyte) making up each chamber result from four consecutive divisions of an oögonial cell located in the germarium. The development of an egg in an ovariole can be subdivided into at least 14 stages, and the number of the egg chambers in each ovary and the proportion of the egg chambers in the various stages depends primarily upon the age of the fly (cf. table 1). Mature eggs (stage 14) in the ovarioles show a Feulgen-positive micronucleus or karyosphere lying free in the oöplasm at the base of the dorsal appendages; whereas the oöcytes of egg chambers in stages 3–13 show a Feulgen-positive nucleolus or karyosome lying in a reticulate nucleoplasm which is surrounded by nuclear membrane. The work of PLOUGH (1917) indicates that genetic recombination has already taken place in chromosomes of the oöcytes in the most anterior egg chambers. Diakinesis and metaphase I occur subsequent to the time the egg leaves the ovariole (SONNENBLICK 1950; FAHMY 1952). Because the anatomical arrangement of the germ cells in the tubes is such that the most mature cells are closest to the uterus, successive batches of eggs laid subsequent to radiation exposure represent cells which on the average were at increasingly early meiotic and premeiotic stages at the time of treatment. Characteristically, the rates of mutation observed are highest in the germ cells first produced, and a decline is noted in the frequency of mutation in successive batches of germ cells (MAVOR 1924; PATTERSON, BREWSTER and WINCHESTER 1932; MAINX 1940; GALL 1950; MULLER, VALENCIA and VALENCIA 1950; KING 1952; NOVITSKI 1953; PARKER 1953; LÜNING 1954; MULLER and HERSKOWITZ 1954; HERSKOWITZ

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TABLE 1

Average number and distribution with respect to stage of developing eggs in the ovaries of flies of different ages

Age (hrs) of virgin female	Stage of development									Total
	1	2	3[x]	4[x]	5[x]	6[x]	7[x]	8[x]–13[x]	14[+]	
0–1	24	25	24	20	16	20	20	0	0	149
96–97	24	25	25	20	10	24	24	0	24	176

[x] Karyosome stages.
[+] Karyosphere stages.

1954; KUTSCHERA 1954; PARKER 1954, 1955; KING and WOOD 1955; GLASS 1955a, b; KING 1955a, b; and GLASS 1956). This paper will describe a study which was initiated to determine whether or not the rates of various types of radiation-induced mutations eventually decline to zero.

GENERAL METHODS

A technique has been described (KING 1955b) which allows collection of almost all eggs laid by female flies over an extended period of time. This technique permits study of the effect of irradiation upon fecundity and fertility, and since the F_1 generation is obtained, studies of hereditary changes are also possible. The germ cells studied were produced by control and treated (2000r Co^{60} γ-rays delivered in ca. 40 seconds) female flies during periods up to 40 days subsequent to the time of treatment (T_0). All cultures were maintained at 25°C. Control and treated females were compared with respect to (1) fecundity (the average number of eggs laid per female for a given time interval); (2) the frequency of dominant lethals in samples of eggs laid (the induced dominant lethal frequency, L, is given by the equation $L = (a - b)/(1 - b)$ where the frequencies of eggs failing to hatch in treated and control series are a and b, respectively); (3) the frequency of X chromosome losses in eggs free of dominant lethals (the induced rate of X losses, $X - L$, is given by the equation $X - L = 2(p_2 - p_1)$, where p_1 and p_2 are the frequency of X0 males in the F_1 generation of the control and treated series, respectively); (4) the ratio of ring to rod X chromosomes in the F_1 offspring of ring-rod heterozygous females; and (5) the frequency of sex-linked, recessive, lethal mutations (in cells containing X chromosomes, but free of dominant lethal and sterility mutations). It is assumed that dominant lethal mutations and X chromosome losses result from gross chromosomal aberrations following chromosome breakage; whereas the sex-linked, recessive, lethal category of mutant is made up by a proportionally large number of point mutations.

EXPERIMENT 1

Methods

Criteria 1–4 were studied in the first experimental series. Here freshly hatched females of the genotype $X^{C2}/M5$ (sc^{81} *B InS apr* sc^{8})[4,5] were etherized, placed in

[4] See BRIDGES and BREHME 1944 and KING 1955a for descriptions of the mutants used.
[5] The X^{C2} stock was checked periodically to insure that the ring X-chromosome was still pres-

plastic tubes, irradiated, reëtherized, and placed in mesh-covered, plastic hoops with untreated males of the genotype $ac^3\ apr/sc^8$.Y. The hoops (each with 50 pairs of flies) were transferred daily for 35 consecutive days to petri dishes containing fresh medium. The number of eggs deposited through the mesh onto the medium was counted, and a day later the number of unhatched eggs was determined. Appropriate controls were maintained. Records were kept of the number of P_1 females surviving in the hoops and fresh males were added once a week. The phenotypic composition of the surviving adult F_1 population was determined. Four classes of regular and one class of exceptional offspring were obtained[6] (regular — $M5/sc^8$.Y (apricot, Bar males), X^{C2}/sc^8.Y (+ males), $M5/ac^3apr$ (apricot, heterozygous Bar females), and X^{C2}/ac^3apr (+ females); exceptional — $ac^3\ apr/0$ (sterile achaete, apricot males).[7] To obtain data for cells treated as mature eggs, irradiated and control 3–4 day old females were studied. Zero-1 day old females were etherized, placed in plastic tubes, stored 3 days, irradiated, and then placed unetherized with males in hoops. Mating generally occurred at once and the eggs laid the first day after treatment were studied for criteria (2), (3) and (4).

Results

The results of experiment 1 may be summarized as follows. Over the 35-day period studied for the initial experimental series, the control and treated females showed no significant difference in fecundity (cf. fig. 1). Under the experimental conditions an average of six eggs was laid daily by each female. No eggs were laid the first day. After inspection of the data, the conclusion was reached that the hatchability of eggs laid by control and irradiated flies appeared to be the same subsequent to the thirteenth day. A standard analysis of variance showed that no significant difference in hatchability occurred from day to day for days 2–35 (eggs 1–205) in the control and days 13–35 (eggs 71–205) in the irradiated series. The average rates for these intervals were computed, and the treated and control values were shown not to differ significantly. The conclusion reached is that in both cases approximately the same proportion (11%) of all eggs laid fail to hatch for one of the following reasons: (a) they are unfertilized, (b) they die because they were improperly formed during oögenesis, or (c) they die during embryogenesis because of aneuploidy following

ent. Ring X-chromosomes were observed in larval salivary gland smears and crossing over studies on ring/rod heterozygotes showed the expected suppression of single crossover classes. The progeny recovered belonging to "single crossover classes" amounted to one-twelfth those normally found for the regions *apr-sn* and *sn-B*.

[6] The separation of *apr* and *B* occurs following certain types of double exchange between the X^{C2} and M5 chromosomes. Flies belonging to crossover classes occurred rarely (5.3, 7.9, 8.6 and 1.0 per 10,000 for heterozygous Bar females, apricot females, apricot males, and Bar males, respectively). Evidently *Bar*-containing crossover products are lethal (especially when hemizygous) more often than *apricot*-containing crossover products. No significant difference was noted between control and treated flies.

[7] Whenever achaete, apricot males were obtained, they were mated singly to two virgin females. No offspring were ever observed. XO, achaete, apricot males have a viability of 0.7, so that the values obtained for the frequency of XO males are underestimates.

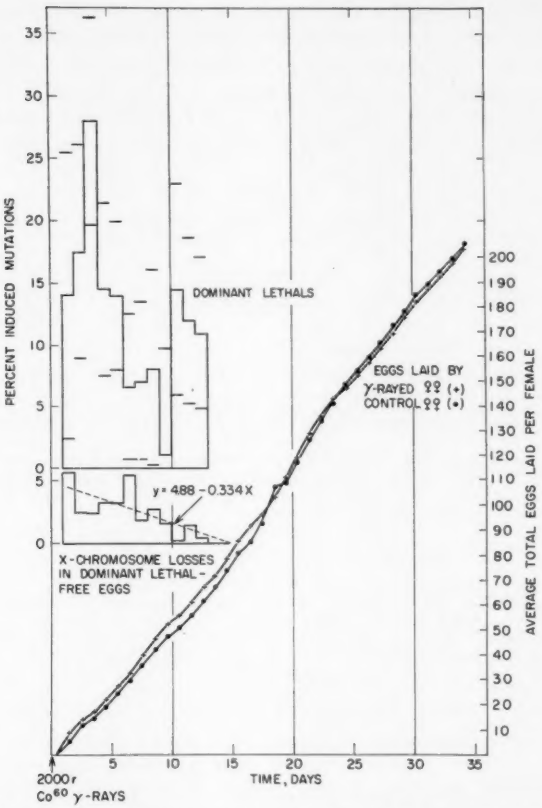

FIGURE 1.—Percent induced mutations and average total eggs produced per female (treated when freshly hatched) as a function of time. The percentage of eggs bearing induced dominant lethal mutations for each daily interval is shown with its 95% confidence limits (calculated from the analysis of variance).

attempted segregation of crossover products between the ring and the rod X chromosomes. With respect to the first 70 eggs laid (days 2 through 12), a statistical analysis showed that the induced dominant lethal rate decreases with time in a non-linear fashion. With respect to the induced rate of viable X chromosome losses, the situation is similar, except that the rate increases with time in a linear fashion (cf. fig. 1). Furthermore, no significant difference exists from day to day for the ring to rod X chromosome ratio for the series; γ-ray (2–12 days), γ-ray (13–35 days) and control (2–35 days). The average values for each series were not significantly different from each other. These data are summarized in tables 2 and 3 (columns B and C). Similar

studies on the eggs laid within one day after treatment by 3–4 day old females gave very different results (cf. tables 2 and 3—column A). The dominant lethal rate was much higher and so was the frequency of X0 males. The ring/rod ratio was somewhat lower, but the sample size was small because so few eggs hatched in this series. It is seen that our results differ from those of FABERGÉ (1952), who made no attempt to differentiate between mutation rates in more and less mature cells.

TABLE 2

Mutation as a function of cell class

P_1 female	Cell class	Series	Frequency of eggs not hatching $\pm s_{\bar{x}}$	N	Frequency of XO males⊕ or X-recessive⊗ lethals	N	Average induced frequency of	
							Dominant lethal mutations = L	X-losses⊕ or X-recessive lethals⊗
Rod / Ring	A	γ	0.9924 ± 0.0170 (a)	2,485	0.0510 ⊕ (p_2)	764	0.986 ±	0.0972 ± ⊕
		C	0.4710 ± 0.281 (b)	3,107	0.0024 ⊕ (p_1)	1,236	0.032 (s_L)	0.0162 (s_d)
	B	γ	0.2232 ± 0.0129	42,140	0.0157 ⊕	17,510	0.129 (cf. figure 1)	0.0256 (cf. ⊕ figure 1)
		C	0.1079 ± 0.0101	36,960	0.0029 ⊕	16,397		
	C	γ	0.1265 ± 0.0084	59,644	0.0035 ⊕	29,137	0	0 ⊕
		C	0.1065 ± 0.0085	62,908	0.0032 ⊕	36,066		
Rod / Rod	A′	γ	0.7821 ± 0.0146	2,605	0.0226 ⊗	1,634	0.636 ±	0.0226 ± ⊗
		C	0.4010 ± 0.0267	2,597	0 ⊗	A′ + B′ pooled 1,778	0.029	0.0037
	B′	γ	0.2536 ± 0.0143	2,937	0.0109 ⊗	1,288	0.174 ±	0.0109 ± ⊗
		C	0.0969 ± 0.0114	2,380	0 ⊗	A′ + B′ pooled 1,778	0.019	0.0029
	C′	γ	0.2161 ± 0.0073	3,171	0.0125 ⊗	1,435	0	0.0125 ± ⊗
		C	0.1985 ± 0.0072	3,031	—	—		0.0029

A = Cells produced 0–1 days after T_0 by females 3–4 days old at T_0. A′ = 1st 26–30 eggs produced 0–4 days after T_0 by females 3–4 days old at T_0.

B = Cells produced 2–12 days after T_0 by females freshly hatched at T_0. B′ = 1st 29–33 eggs produced 12–15 days after T_0 by females 3–4 days old at T_0.

C = Cells produced 13–35 days after T_0 by females freshly hatched at T_0. C′ = cells produced 30–40 days after T_0 by females 3–4 days old at T_0.

γ = P_1 females given 2000r Co^{60} γ-rays in 40 seconds. C = P_1 females not treated.

Ring/Rod = $X^{C}2$/M5 females × ac^{s} apr/sc^{s}. Y males.

Rod/Rod = Oregon R females × M5 males.

T_0 = Time of irradiation. X-losses = 2 ($p_2 - p_1$).

N = The size of the population (of eggs, F_1 offspring, or X chromosomes) examined.

$$s_{\bar{x}} = \sqrt{\frac{pq}{N}} = \sqrt{\frac{\Sigma x^2 - \left(\frac{\Sigma x}{N}\right)^2}{N(N-1)}};$$

$$s_d = \sqrt{\frac{p_1q_1}{N_1} + \frac{p_2q_2}{N_2}};\ L = \frac{a-b}{1-b};\ s_L = \sqrt{\left(s_{\bar{x}_a} \cdot \frac{1}{1-b}\right)^2 + \left(s_{\bar{x}_b} \cdot \frac{(1-a)}{(1-b)^2}\right)^2}$$

TABLE 3

X chromosome composition of F_1 population resulting from the cross $X^{C2}/M5$ females × $ac^s apr/Y$: sc^s males

Cell Class	Series	% of total F_1 flies from the regular classes†					
		Females		Males		F_1 ring/ rod ratio	
		Ring	Rod	Ring	Rod	Females	Males
A	γ	23.3	28.3	23.0	25.4	0.824	0.908
	C	25.8	29.1	22.4	22.7	0.886	0.986
B	γ	26.9	29.3	22.5	21.2	0.918	1.061
	C	27.6	28.0	22.8	21.7	0.986	1.051
C	γ	27.7	28.1	22.1	22.0	0.986	1.005
	C	27.1	29.2	21.7	22.1	0.928	0.982

† Cf. table 2 for explanation of abbreviations and data on the frequency of XO males in the total F_1 and the total number of F_1 offspring in each series.

EXPERIMENT 2

Methods

The second series of experiments involved the determination of the frequency of X chromosomal, recessive, lethal mutations (criterion 5) in germ cell populations whose dominant lethal rate was known. Three to four day old Oregon R females were divided into two groups, one of which was irradiated. Females were mated individually with untreated M5 males in mesh-covered hoops. Records were kept of the fecundity and fertility of the flies at a series of time intervals subsequent to T_0. The survivors from the eggs laid during each interval developed into F_1 adults, and F_1 heterozygous Bar females were backcrossed to Oregon R males and then isolated singly in small culture bottles (¾ oz creamers). The population of creamers was scored for lethals (cultures with no + males) when the F_2 generation emerged. Records were kept which enabled us to tell which F_2 cultures originated from the same P_1 females. In this way it was possible to determine if the same P_1 female produced more than one lethal. The frequencies for recessive lethal mutation recorded are therefore free from errors due to duplicated lethals, but may be underestimates if many duplicated non-lethals were included.

Results

The results of the second group of experiments are summarized in columns A′, B′ and C′ of table 2. It is obvious that those cells (class C′) produced 30–40 days after T_0 by irradiated females show no difference in hatchability from those produced by untreated females. However, they are characterized by a rate of X chromosomal, recessive, lethal mutations which is not significantly different from those produced

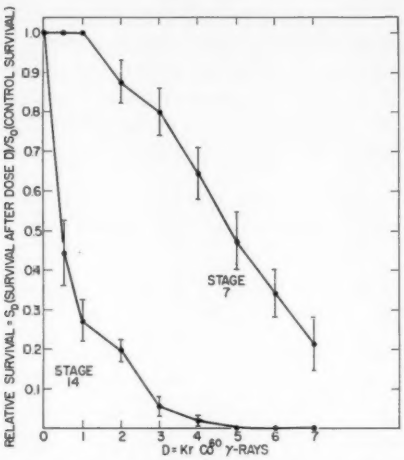

FIGURE 2.—The relation between relative survival and dose for oöcytes belonging to stages 7 and 14. Each point is bracketed by its 95% confidence limits

$$\left(\frac{S_D}{S_0} \pm 1.96 \sqrt{\frac{1-a}{1-b}\left[\frac{a}{N_a(1-b)} + \frac{b}{N_b}\right]}\right).$$

The frequencies of eggs failing to hatch in treated and control series are a and b, respectively.

12–15 days after T_0. It is also obvious that the first eggs produced by 3–4 day old females have a higher induced rate of dominant lethal mutations, if the female is a ring/rod heterozygote, than if the female had two rod-X-chromosomes. PONTECORVO (1942) has summarized the reasons why the chromosomal products resulting from the reunion of broken ends of ring chromosomes should be lethal to the eggs containing them. The fact that we observe the difference described above indicates that there is a genetic basis for the failure of treated eggs to hatch, and consequently the term "dominant lethal" is justified.

EXPERIMENT 3

Methods

In order to obtain further information about the A and B categories of cells in the ovaries of *Drosophila melanogaster*, we decided to determine the dose-response relations for dominant lethal mutations induced in cells belonging to stages 14 and 7. Eight exposures to Co^{60} γ-rays (ca. 50r/sec) were used: 500, 1000, 2000, 3000, 4000, 5000, 6000, and 7000r. Virgin, Oregon-R females (0–1 hour and 96–97 hours old) were treated and mated to untreated males. Care was taken to collect less than the first thirty eggs laid subsequent to treatment, and in this way we sampled in most cases only the posterior egg in each ovariole. It is apparent from table 1 that we were therefore determining the induced rate of dominant lethals in cells treated at stages 7 (P_1 females 0–1 hour old) and 14 (P_1 females 96–97 hours old).

TABLE 4

Egg hatch vs dose

Dose (KR)	Stage 14		Stage 7	
	N	S	N	S
0	941	0.849	1,478	0.777
0.5	172	0.378	326	0.807
1.0	314	0.232	404	0.800
2.0	940	0.171	1,008	0.681
3.0	431	0.049	508	0.618
4.0	719	0.019	357	0.501
5.0	99	0.000	215	0.367
6.0	132	0.000	260	0.265
7.0	127	0.000	234	0.167
Total.....	3,875		4,790	

N = total eggs observed.
S = frequency hatching.

Results

The dose-response relations obtained from experiment 3 are shown in table 4 and figure 2. The dose-response relations for stage 14 oocytes and for stage 7 oocytes can be described by a one event curve and by a multiple event curve, respectively (cf Parker 1955). We assume that dominant lethality requires isochromatid breakage in at least two different bivalents followed by sister union of the broken chromatids and passage of the fused bivalents so produced to opposite poles at anaphase 1. The "event" is visualized as the passage of an electron tail through the genetic material. In the case of the mature egg a single electron tail traversing the densely packed bundle of chromatids contained in the karyosphere presumably causes multiple isochromatid breaks; whereas multiple tails are required in the case of the stage 7 oocyte nucleus to produce the same effect. The resulting anaphase 2 chromatid bridges tie the terminal ootid nuclei to the inner ootid nuclei with the result that the female pronucleus receives an aneuploid chromosome complement.

DISCUSSION AND CONCLUSIONS

Our conclusions are summarized in table 5. The germ cells studied in experiments 1 and 2 can be subdivided into three categories (A, B and C) on the basis of their mutational responses. Cells of class A show a high rate of dominant lethal mutation and of X chromosome loss and recessive mutation. When treated, these cells were stage 14 primary oöcytes containing a free karyosphere. Cells of class B show lower rates of all three types of genetic change. Under the experimental conditions employed, these cells at the time of treatment were generally the posterior-most three egg chambers in the ovarioles of the exposed females. Therefore, these cells were less mature chambers of varying size (stages 3–7 were represented) with their oöcytes characterized by a karyosome-containing nucleus. Since the fecundity of treated females was unaltered, and since the primary oöcytes of similarly treated flies ap-

TABLE 5

The correlation of mutational behavior with cellular morphology

	Class of induced mutants	Germ cell class		
		A	B	C
Mutational response to 2000 r of gamma rays	Dominant lethal mutations	++++++	+	0
	X chromosome losses	+++	+	0
	X chromosome recessive lethal mutations	++	+	+
Stages of oogenesis represented		14	3–7.	1, 2 and oogonia
Nuclear morphology		Karyosphere	Karyosome	No karyosome
Genetic recombination occurs		Prior to	Prior to	During and after

peared cytologically normal[8], it is unlikely that mutated oöcytes in egg chambers are prevented from maturing and being laid as eggs. Therefore, the difference in sensitivity to mutation between cells of groups A and B must be real and not a result of germinal selection. The cells of group C show no induced dominant lethals and X chromosome losses, but significant numbers of sex-linked recessive lethal mutations. The class C group of cells includes oögonia, each of which must subsequently undergo four consecutive divisions to produce the sixteen components of the egg chamber. Possible oögonia containing genetic lesions which would later be detected as dominant lethals or X losses cannot produce a normal cyst of sixteen orthoploid cells. Perhaps such cells are resorbed at a very early stage and replaced by viable cells. If this actually occurs, one would expect to see pycnotic nuclei in the germaria of treated females. Our cytological studies[8] support this suggestion. However, the class C group of germ cells must *also* contain developing eggs which at the time of treatment had passed the four divisions and were in stages 1 and 2. The above considerations cannot apply to these cells. It is also possible that a damaged, potential oöcyte nucleus may be resorbed and its place is taken by an undamaged nucleus which would normally differentiate into a nurse cell nucleus. However, we find no evidence of chambers with less than the normal number of nurse nuclei in irradiated females[8]. The foregoing statement applies only to females which received radiation exposures of 2000r or less. At higher doses one sees that the nuclei of the oöcyte, nurse cells and follicle cells lose their ability to stain darkly following the Feulgen procedure, egg chambers are found which appear normal except that they contain more or less than 15 nurse cells, and degenerating chambers are often observed. Most germaria degenerate completely following exposure to 6000r of γ-rays or more. At lower doses the ovary often recovers, but ovarian tumors appear subsequently (KING, RUBINSON and SMITH 1956).

The simplest explanation for the failure to recover dominant lethals or X losses from stage C cells is that (1) the probability of chromosome breakage is very low or

[8] A manuscript describing these studies is in preparation.

(2) that the probability of restitution very high in chromosomes at early stages of oögenesis; or to put it more specifically, in chromosomes prior to the karyosome stages. If explanation (1) is correct, then sex-linked, recessive, lethal mutations recovered from class C cells may not have required chromosome breakage for their origin. If explanation (2) is correct then the high probability of restitution may be in some way related to the nuclear conditions which allow genetic recombination.

Dominant lethal mutations and X chromosome losses are taken as a measure of chromosome breakage in these experiments. The data show that the sensitivity of the germ cells to radiation-induced chromosome breakage varies during oögenesis in *Drosophila melanogaster*. Unlike dominant lethal mutations and X chromosome losses which under the experimental conditions are not recovered in significant numbers from oöcytes which were at stages of oögenesis earlier than the karyosome stages, sex-linked, recessive, lethal mutants are found in eggs which were in these and even more immature stages at the time of treatment[9]. It is probable, therefore, that mutations of this category (mostly point mutations) are *never* completely eliminated from the germ tract, whereas dominant lethals and X losses (mostly the result of gross chromosomal aberrations) are eliminated once the germ cells (which at the time of treatment were karyosome-containing oöcytes) complete development and are laid.

When attempting to assess the genetic damage from radiation exposure, one must take into account the interval between the exposure and fertilization in order to predict the type of genetic change which is likely to persist. In female *Drosophila melanogaster* and higher animals the dominant lethal category of genetic changes is the easiest to study. In the fruit fly this category of mutant is also the class most readily eliminated from the ovary, whereas recessive lethal mutants persist. Therefore, if no significant difference in the rate of dominant lethal mutations were noted between females of irradiated and non-irradiated populations, an investigator could not conclude that the exposure to ionizing radiation failed to damage the germinal material of the members of the irradiated population unless the attendant conditions made it unlikely that in the average female a large proportion of the germ cells bearing dominant lethals was naturally eliminated before fertilization occurred.

SUMMARY

In *Drosophila melanogaster* the sensitivity of the germ cells to radiation-induced chromosome breakage (measured by dominant lethality and X chromosome loss) varies strikingly during oögenesis. These changes are correlated with the cytology of the developing primary oöcytes. Following a single exposure to 2000r of Co^{60} γ-rays (delivered in 40 seconds) such gross chromosomal aberrations are quickly eliminated, but recessive, lethal mutations appear never to be completely eliminated from the germ tract.

ACKNOWLEDGMENTS

DR. PHELPS CRUMP conducted a portion of the statistical analysis of the data and outlined the other procedures used. Exposures to Co^{60} γ-rays were made by R. N. CHAPMAN. PROFESSOR H. J. MULLER kindly supplied the necessary stocks

[9] A mutant cluster occurred in the C group of germ cells which indicates that a mutated oögonium may give rise to duplicate, sex-linked, recessive, lethal mutants.

for synthesis of the breeding systems used. The authors are indebted to DRS. D. F. POULSON and D. R. PARKER for their thoughtful criticisms of this paper.

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CROSSING OVER IN MAIZE IN THE PRESENCE OF THE TRANSPOSABLE FACTORS ACTIVATOR AND MODULATOR

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CONSIDERABLE experimental probing, much of it on an empirical basis, may be necessary before the novel class of genetic factors in maize to which Activator (*Ac*) and Modulator (*Mp*) belong can be viewed in proper theoretical perspective. The present experiment was undertaken on the assumption that because *Ac* and *Mp* may markedly increase chromosome breakage, either directly or indirectly, they might influence crossing over also. Furthermore, there is direct evidence that genetic elements of this kind may influence crossing over. McCLINTOCK (1953a) examined 37 separate cases of mutation to the sh_1 (shrunken endosperm) phenotype in an Sh_1 stock carrying Dissociation (*Ds*) to the left of, but near, the Sh_1 locus. The mutants were found to vary widely both in number of reversions to Sh_1 and in effect on crossing over in the vicinity of the sh_1 locus. The heterogeneity of the latter effects is noteworthy.

No action of *Ac* and *Mp* on crossing over was found in the present test. A negative result is not without interest, however, in view of the obscurity surrounding the intimate relation between these readily transposable factors and the firmly ordered genes.

Activator (McCLINTOCK 1950) and Modulator (BRINK and NILAN 1952) are similar elements, and eventually may prove to be identical. They were discovered in unrelated maize strains, and have been shown to have certain well-defined properties in common. Satisfactorily controlled tests for identity, however, have not yet been completed, and so a distinction between them currently is being maintained.

Modulator has been studied mainly as a component of the highly unstable variegated pericarp allele, P^{vv} (BRINK and NILAN 1952; BRINK 1954). Commonly, in about five per cent of the cases in plants heterozygous for variegated pericarp, P^{vv} mutates to P^{rr} (self color) as a result of transposition of Modulator from the *P* locus, usually to another position in the genome. BARCLAY and BRINK (1954) found that *Mp*, when present in the same nucleus with Dissociation (*Ds*), promotes the *Ds* type of chromosome breakage also. It has been shown more recently (FRADKIN and BRINK 1956) that, in strains free of known *Ds*, Modulator increased three- to five-fold mosaicism for endosperm marker characters in two out of four chromosome regions tested. Evidence was adduced that the principal factor causing the mosaicism was chromosome breakage. Tests for chromosome breakage at the variegated pericarp locus, however, have given negative results.

McCLINTOCK (1956) has demonstrated that Activator, as an autonomous unit at the bronze (*bz*) locus, renders *bz* unstable. *Ac* in this position may also cause local chromosome breakage. She had previously shown in her ingenious analysis of Activator and Dissociation that *Ac* promotes chromosome breakage at *Ds* with high frequency in certain strains (McCLINTOCK 1951, 1953b).

MATERIALS AND METHODS

The short arm of chromosome 9, marked at the three loci conditioning the endosperm characters *sh* (shrunken), *bz* (bronze), and *wx* (waxy), were chosen for testing the effect of Activator and Modulator on crossing over. We are indebted to DR. MCCLINTOCK for the stock of mutable bronze, *bz*^{m}(*Ac*), used. The standard map distances for these loci are about *sh* 1.6 *bz* 18 *wx* (RHOADES 1952; HUTCHISON 1922), waxy being closest to the centromere (MCCLINTOCK 1931). The plants were homozygous for the dominant complementary genes A_1, A_2, C, and R, thus providing the aleurone color genotype necessary for the expression of *bz* and *Bz* (purple).

Unlike *Ac*, which was situated at the bronze locus within the region in which crossing over was measured, Modulator was represented as a component of the variegated pericarp allele on chromosome 1. The dosage effects of *Ac* and *Mp* are parallel (BARCLAY and BRINK 1954) and although the relation between them in this respect has not yet been clearly resolved there is evidence (see later) that they interact with each other in much the same fashion. There were grounds for expecting, therefore, that if *Ac* altered the frequency of crossing over in the vicinity of the bronze locus *Mp*, situated on another chromosome, might modify the effect.

Four groups of plants were prepared for the tests on crossing over by placing the pollen of a single *sh*, *bz*^{m}(*Ac*), *wx*, no-*Mp* individual on the silks of highly inbred and near-isogenic *Sh Bz Wx*, 1 *Mp* (medium variegated pericarp) and *Sh Bz Wx*, no-*Mp* (colorless pericarp) plants. These F_1 groups were characterized with reference to *Ac* and *Mp* as follows:

Group	Composition	Pericarp	Bronze
(a)	1 *Mp*, 1 *Ac*	variegated	mutable
(b)	no-*Mp*, 1 *Ac*	colorless	mutable
(c)	1 *Mp*, no-*Ac*	variegated	stable
(d)	no-*Mp*, no-*Ac*	colorless	stable

The variegated pericarp and mutable bronze phenotypes provided definitive evidence for the presence of *Mp* at the *P* locus and *Ac* at the bronze locus. Classes (c) and (d) arose as spontaneous mutants from *bz*^{m}(*Ac*) to stable bronze. The change, which involves the loss of *Ac* from the *bz* locus, occurred with the frequency of about 15 per cent in this stock. Note should be taken of the fact that the stable allele resulting from the mutation usually is bronze, not purple (cf. MCCLINTOCK 1956).

The control plants, class (d), which were free of both *Ac* and *Mp* at any position in the genome were identified in the F_1 population by testcrossing each individual with *I Ds* pollen (*I* = aleurone color inhibitor, allelic to *C*). Only plants which bore two fertile ears were used in the experiment. Pollen from homozygous shrunken, bronze (stable), waxy individuals was applied to the main ear, which then served as the source of the data on crossing over. *I Ds* pollen was used on the second ear, *Ds* being located in the standard position in chromosome 9, proximal to *I*. Twelve plants were found in which the aleurone showed no *I* to *C* mottling such as would

have occurred had either *Ac* or *Mp*, or both, been present in the genome to promote chromosome breakage at the *Ds* locus.

It was originally hoped to exclude plants carrying transposed Modulator from group (b) and those carrying transposed Activator from group (c) by the *I Ds* testcross. This proved impracticable. Two doses of *Ac* or *Mp* entering the 3*x* primary endosperm nucleus by fusion of the polar nuclei (*x*) in testcrosses involving *Ds* often so delay *Ds* chromosome breaks in ontogeny that the genetically mottled and normal kernel classes overlap. Qualitatively, the test is effective, but the ratio of mottled to non-mottled kernels often can not be determined with sufficient accuracy to distinguish between the presence of one and two doses of *Ac* or *Mp*. Groups (a), (b), and (c), therefore, were identified on the basis of the pericarp and aleurone phenotypes only. Occasional plants among them may carry supernumerary *Ac* or *Mp* units at loci other than *bz* and *P*, respectively.

The 10 plants in group (a) all had light variegated pericarp, the phenotype expected if *Ac* has the same action on expression of the P^{vv} allele as a transposed Modulator (BRINK and NILAN 1952). The five plants in group (c), a stable bronze class, were medium variegated, an observation which, by the same criterion, shows that *Ac* was absent from the genome. One mutable bronze plant occurred, however, which had medium, rather than light, variegated pericarp. Evidently the *Ac* factor at the *bz* locus in this case had undergone some change affecting its action on the P^{vv} allele. The data on crossing over from the exceptional individual were not included in the computations.

EXPERIMENTAL RESULTS

The crossover data obtained are summarized in tables 1, 2 and 3. The percentages of crossing over given in columns 4 and 5 of table 1 include both single and double crossovers. The latter also are entered separately in column 6.

The average amounts of crossing over shown by the population of 4974 kernels on the 12 ears comprising the control group (no-*Ac*, no-*Mp*) were 3.03% and 14.41% for the *sh-bz* and *bz-wx* regions, respectively. These frequencies are only slightly different from the standard values cited earlier.

The corresponding values for the 10 ears segregating for mutable bronze, $bz^m(Ac)$, with *Mp* probably absent for the genome, are 3.21% and 15.05%. Only five ears, bearing 1894 kernels, were available in the group segregating Modulator, Activator being absent from the *bz* locus and presumably from the genome. The crossover values in this case were 2.34% and 15.00% for the two respective regions. Finally, the 10 ears segregating both Activator at the *bz* locus and Modulator at the *P* locus showed 3.42% crossing over between *sh* and *bz* and 14.61% between *bz* and *wx*.

The mean crossover values for the two regions for each class of plants together with the differences between means after pairing on the basis of presence and absence of *Ac* and *Mp* are shown in tables 2 and 3. Considered in relation to their respective standard errors none of the differences is statistically significant. The present experiment affords no evidence, therefore, that Activator situated within the region tested or Modulator located on another chromosome alters the frequency of crossing over.

TABLE 1

Crossing over in the sh bz wx *region in chromosome 9 in the presence and absence of Activator at the* bz *locus and in the presence and absence of Modulator at the* P *locus in chromosome 1*

1	2	3	4	5	6
Class of plants	Ear number	Number of kernels	Percent crossing over		% double crossovers
			sh-bz	*bz-wx*	
1 *Mp*, 1 *Ac.*	F420–4	377	2.9	14.5	0.5
	F420–36	407	4.7	14.5	1.0
	F420–42	523	2.1	14.4	0.7
	F421–4	295	3.8	16.0	0.7
	F421–7	299	4.3	14.7	0.3
	F421–22	360	3.1	13.7	0.6
	F421–25	482	2.7	14.3	0.0
	F421–45	434	3.0	12.0	0.7
	F421–46	441	3.2	17.0	0.7
	F421–74	360	4.4	15.0	0.8
Total and means		3978	3.42	14.61	0.60
no Mp, 1 *Ac.*	F424–1	302	3.3	14.6	0.7
	F424–3	587	3.9	16.8	0.3
	F424–4	343	4.1	15.8	0.6
	F424–17	409	2.0	15.6	0.0
	F424–19	437	2.7	13.2	0.2
	F424–27	486	3.5	14.0	0.0
	F425–19	498	3.0	16.9	0.6
	F425–22	380	2.6	16.3	0.8
	F425–24	593	3.4	13.8	0.5
	F425–30	274	3.6	13.5	0.0
Total and means		4309	3.21	15.05	0.37
1 *Mp*, no-*Ac.*	F420–9	396	2.0	14.9	0.0
	F420–61	381	2.1	14.7	0.0
	F421–23	428	3.3	16.6	1.2
	F421–24	300	2.0	15.7	0.0
	F421–50	389	2.3	13.1	0.0
Total and means		1894	2.34	15.00	0.24
no-*Mp*, no-*Ac.*	F424–5	379	4.0	12.9	0.0
	F424–7	129	2.3	17.1	0.0
	F424–9	319	6.6	12.8	0.6
	F424–10	379	4.0	17.7	1.1
	F424–37	592	3.5	14.4	0.0
	F424–41	458	2.4	12.4	0.0
	F424–49	587	1.7	15.5	0.0
	F425–5	195	1.0	12.3	0.0
	F425–12	717	4.2	16.3	0.7
	F425–14	409	3.1	12.2	0.2
	F425–17	345	1.2	13.6	0.0
	F425–18	465	2.4	15.7	0.0
Total and means		4974	3.03	14.41	0.22

TABLE 2

Mean percentages of crossing over between sh *and* bz *in the presence and absence of* Ac *and* Mp

	0 *Mp*	1 *Mp*	Mean	Effect of *Mp*	S_d
0 *Ac*	3.03	2.34	2.69	−0.69	0.40
1 *Ac*	3.21	3.42	3.32	0.21	0.24
Mean	3.12	2.88	3.00	−0.24	
Effect of *Ac*	0.18	0.08	0.13		
S_d	0.36	0.26			

TABLE 3

Mean percentages of crossing over between bz *and* wx *in the presence and absence of* Ac *and* Mp

	0 *Mp*	1 *Mp*	Mean	Effect of *Mp*	S_d
0 *Ac*	14.41	15.00	14.71	0.59	0.58
1 *Ac*	15.05	14.61	14.83	−0.44	0.37
Mean	14.73	14.81	14.77	0.08	
Effects of *Ac*	0.64	−0.39	0.13		
S_d	0.52	0.48			

DISCUSSION

It has been found in the present study that the frequency of crossing over in the *sh-bz-wx* region of chromosome 9 in maize was not detectably affected by the presence of Activator at an intermediate locus, *bz*, which is thereby rendered highly unstable. Nor does Modulator, a similar, or possibly identical, element introduced into the genome as a component of the variegated pericarp allele on chromosome 1 influence the amount of recombination in the same region in the presence of either stable or mutable bronze. The tests were made in comparatively homogeneous stocks although not on a scale which would have revealed slight effects.

No definitive conclusions can be drawn from the results of this experiment on the intimate relation of Activator and Modulator to the "gene string". It is evident, however, that crossing over is not necessarily disturbed locally by the presence of one of these elements in heterozygous condition. The frequent chromosome breakage which *Ac* and *Mp* may cause in somatic cells either autonomously or through interaction with *Ds*, or *Ds*-like factors, is not reflected in this case in the orderly process of exchange of homologous chromosome segments occurring at meiosis. The data also show that the mere rendering of a locus unstable by the advent of *Ac* does not necessarily alter the capacity for regular genetic recombination in the adjacent region. It would be rash, however, to generalize in this respect from the results of a test involving a single locus and one particular transposable factor. This and other avenues will have to be more extensively explored before the essential relations of these peculiar chromosomal elements to the mechanics of the cell nucleus are known.

SUMMARY

The effect in maize of Modulator (*Mp*) as a component of the variegated pericarp allele on chromosome 1 and of Activator (*Ac*) at the bronze (*bz*) locus on the fre-

quency of crossing over in the *sh-bz-wx* region in chromosome 9 was studied. The amount of crossing over was measured in the *sh-bz* and *bz-wx* regions in four comparable groups of plants differing in *Ac* and *Mp* content as follows: no-*Ac*, no-*Mp* (control); 1 *Ac*, no-*Mp*; no-*Ac*, 1 *Mp*; and 1 *Mp*, 1 *Ac*. No significant differences in frequency of crossing over between the four groups of plants were found.

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CHROMOSOME SEGREGATION IN DROSOPHILA VIRILIS MALES WITH MULTIPLE SEX CHROMOSOMES[1]

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ONE of the many puzzling aspects of chromosome mechanics is the process of pairing of homologous chromosomes and their disjunction at the first meiotic division. A genetic indication of the intimacy of pairing is the amount of exchange taking place between homologous chromosomes. Where more than two chromosomes share homologies, as in translocation heterozygotes, the correlation between exchange and disjunction of chromosome arms may be measured. Although there is a positive correlation between these two processes in translocation heterozygotes, the relation is not necessarily causative since nonrandom disjunction of chromosomes occurs in the absence of crossing over. For example, STURTEVANT (1936) observed preferential segregation in triplo-IV females of *Drosophila melanogaster* where exchange between homologous fourth chromosomes occurs with a negligible frequency. In addition, COOPER (1946) has shown nonrandom disjunction of the multiple sex chromosomes of *D. miranda* males, and KIKKAWA (1935) obtained evidence that in *D. virilis*, in contrast with *D. melanogaster*, segregation in X/Y/Y males is preferential. Meiotic exchange in males of both species is virtually absent. It appears that disjunction may be related to the amount of pairing but not necessarily through exchange.

KIKKAWA (1935) assumed that a bivalent and a univalent, rather than a trivalent, were formed in X/Y/Y males, whereas STURTEVANT's data (1936) suggested a trivalent association of the three fourth chromosomes. COOPER (1949) showed in his elegant cytological analysis of meiosis in male *D. melanogaster* that the chromosome association invariably observed in X/Y/Y males is a trivalent and in X/Y/Y/Y males a tetravalent. In view of COOPER's earlier interpretation of secondary nondisjunction in female Drosophila (1948) (namely, each arm of the Y has a site that pairs with a region on the X), it is conceivable, although not likely, that multivalent formation in *D. melanogaster* males is related to the two-armed nature of the Y and nonrandom segregation in *D. virilis* males may merely reflect preferential bivalent formation as KIKKAWA proposed, since in this species the Y appears as a rod-shaped chromosome.

A few years ago a more adequately marked Y became available in *D. virilis*, which allowed a study to be made of chromosome association and disjunction in males with multiple sex chromosomes. The results, which are reported in this paper, indicate a complex preferential segregation from an invariable multivalent association. An abstract of these results has been published (BAKER 1954).

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MATERIAL AND METHODS

The synthesis of *D. virilis* males with extra marked Y chromosomes became possible when a mottled female arose from primary nondisjunction in a male carrying $T(Y; 5)pe^{ml}$ (BAKER 1953). The 5^Y chromosome of this translocation carries pe^+ (pe = peach, 5-203) and all the fertility factors of the Y chromosome. This rearrangement involves a break distal to pe^+ in chromosome 5, but, as pointed out to the author by E. B. LEWIS (personal communication), the 5^Y chromosome may not be a reciprocal translocation but rather an insertion of pe^+ in the Y. It could just as logically be designated Y: pe^+ as 5^Y; the former designation will be used in this paper since the new location of pe^+ causes somatic variegation, and an additional break proximal to the locus would then make this case of mottling fall into the usual pattern of break-

TABLE 1

Segregation of multiple sex chromosomes in Drosophila virilis *males*

Type of P_1 male	Sperm of P_1	Classes of X_2 offspring from cross P_1 ♂♂ × wild type ♀♀					Estimated frequency of sperm
		F_1♂ × *pe* ♀			F_1 ♀ × *pe* ♂		
		W ♀♂ P ♀♂ M ♀♂	W ♂♀ P ♀* M ♂	W ♂♀ P ♂♀	W ♀♂ P ♀♂ M ♀♂	W ♀♂ P ♀♂	
X/Y/Y:pe^+;*pe* (5)	Y			x			0.198
	X/Y:pe^+				x		0.198
	Y/Y:pe^+	x					0.129
	X					x	0.129
	Y:pe^+		x				0.173
	X/Y					x	0.173
	Number observed	59	79	76	84	108	
		214 ♂♂ tested			192 ♀♀ tested		
X/Y:pe^+/Y:pe^+;*pe* (23)	X/Y:pe^+				x		0.262
	Y:pe^+		x				0.262
	X					x	0.238
	Y:pe^+/Y:pe^+	x*					0.238
	Number observed	348	384		416	379	
		732 ♂♂ tested			795 ♀♀ tested		
X/Y/Y:pe^+/Y:pe^+;*pe* (4)	X/Y:pe^+				x		0.341
	Y/Y:pe^+	x					0.341
	X/Y					x	0.145
	Y:pe^+/Y:pe^+	x*					0.145
	X					x	0.006
	Y/Y:pe^+/Y:pe^+	x					0.006
	X/Y/Y:pe^+				x		0.003
	Y:pe^+		x				0.003
	X/Y:pe^+/Y:pe^+				x*		0.006
	Y			x			0.006
	Number observed	170	1	2	93	40	
		173 ♂♂ tested			133 ♀♀ tested		

Columns 3 and 4: W, wild type; P, peach; M, mottled.

* A few peach males are produced when variegation goes to completion (see BAKER 1953). Figures in parentheses (column 1) indicate the number of different P_1 males tested of the type indicated.

age and *pe*-variegation in *D. virilis*. It is probable, but not certain, that the complementary chromosome, i.e., the Y^5 or the *pe*-deficient fifth chromosome, was eliminated from the strains before the present experiments were begun.

It was desired to study segregation in three types of males: X/Y/Y: pe^+, X/Y: pe^+/Y: pe^+, and X/Y/Y: pe^+/Y: pe^+. Mottled males of the type X/Y/Y: pe^+; *pe* were formed by crossing X/X/Y: pe^+; *pe* females to *pe* males. Practically all the mottled males from this cross are the desired type since secondary nondisjunction in *D. virilis* females is rare (0.53%, according to KIKKAWA 1932).

Mottled males of the type X/Y: pe^+/Y: pe^+; *pe* were formed by crossing X/X/Y: pe^+; *pe* females with X/Y: pe^+; *pe* males. Not only are mottled males of the indicated type produced by this cross but also X/Y: pe^+; *pe* males. The two types of males were distinguished by progeny testing each male to *pe* females. From this testcross, males of the desired type will produce both mottled sons and mottled daughters, whereas only sons of the other type of male will be mottled.

From the cross X/X/Y: pe^+; *pe* females to X/Y/Y: pe^+; *pe* males, the following four types of mottled males are produced: X/Y: pe^+, X/Y/Y: pe^+, X/Y: pe^+/Y: pe^+, and X/Y/Y: pe^+/Y: pe^+. None of the types can be distinguished phenotypically, but each can be differentiated in the X_2 of crosses (see table 1) made to determine the segregation pattern. The first of the four types of males listed may be distinguished from the other three in that no mottled females will appear in his progeny. An examination of table 1 will indicate the characteristic classes of X_2 offspring that can be used to differentiate the remaining three types of mottled males. Thus it was possible to obtain segregation data on known X/Y/Y: pe^+/Y: pe^+ males. It is interesting to note that these males are fertile, whereas in *D. melanogaster* over 97% of males with three Y chromosomes are sterile (COOPER 1949). Either there is a species difference in the effect of extra Y's on sterility, or the Y: pe^+ chromosome is not an insertion; thus three complete Y's are not present in *D. virilis*.

RESULTS

Segregation patterns of the four types of P_1 males denoted in table 1 were disclosed by crossing each male individually to a wild type female. The type of segregation pattern determines the relative frequency of F_1 offspring of the various chromosomal constitutions; these, in turn, were ascertained by crossing the F_1 offspring individually to *pe* flies. The phenotypic classes in the offspring of this cross are distinctive for the different chromosomal constitutions of the F_1, as can be seen by examination of table 1. For example, of the six types of sperm formed by a P_1 male of the type X/Y/Y: pe^+, three produce F_1 males that give characteristically different X_2 offspring, and the other three types produce F_1 females in which one of the three chromosomal constitutions can be differentiated in the X_2. It should be noted that the determination of the relative frequency of the types of P_1 sperm depends only on the qualitative recognition of phenotypic classes in the X_2 and not on the relative frequency of mutant classes. Thus the data, in contrast to much of the previous work on segregation, are not biased because of viability differences.

The data from which the segregation patterns are derived are presented in the "Number observed" rows of table 1. A number of P_1 males of each type were tested; however, only the lumped data are presented because chi-square tests provided no

evidence of heterogeneity among the males of any given type. The basic data are converted into frequencies of the different sperm types and recorded in the last column of the table. One assumption inherent in this calculation is that complementary types of gametes are equally likely to take part in fertilization; i.e., there is no differential meiotic loss of chromosomes or unequal functioning of gametes. This qualification is valid, since complementary classes of gametes produce individuals of opposite sex and no deviation from a 1:1 sex ratio was observed in the F_1 offspring. In estimations of gamete frequency, the data from F_1 males and females of a particular segregation class were given equal weight. Where two or more segregation types produced the same phenotypic class, the frequency was estimated by dividing the number of offspring of this class in proportion to the distribution of the complementary sperm types found in the opposite sex. One arbitrary assumption had to be made in estimations of the gamete frequency in the X/Y/Y: pe^+/Y: pe^+ males since there are five unknowns (the types of segregation) and only four independent equations. This assumption, that the X chromosome disjoins from the other three with the same frequency that the Y disjoins from the remaining three, is a reasonable approximation. Notwithstanding, one would expect this type of 3-1 disjunction to be at least similar in frequency to the two other types of 3-1 disjunction (which are less than 1%); therefore, the absolute validity of the approximation will have little bearing on the estimated values of the segregation types.

The last column of table 1 shows that the segregation patterns are not predictable from one type of male to another. For example, one might think, on *a priori* grounds, that the pattern in X/Y/Y: pe^+ males would be similar to that found in X/Y: pe^+/Y: pe^+ males. However, in the former the two Y's segregate from each other 74% of the time, whereas in the latter the two Y's separate with a frequency of about 48%. Therefore, the segregation pattern is a function of each particular association of chromosomes.

DISCUSSION

An understanding of the manner in which multiple sex chromosomes are associated is essential for interpretation of the segregation data. The importance of this association becomes clearly evident if an attempt is made to interpret the data in terms of synapsis of the three homologs, in the manner usually assumed in the past; i.e., by formation of a bivalent and a univalent that passes at random. KIKKAWA (1935), applying such an interpretation to his data on segregation in X/Y/Y males of *D. virilis*, concludes that the two Y's pair 58% of the time whereas the X pairs with each one of the Y's with a frequency of 21%. On the same interpretative basis, the data from the X/Y/Y: pe^+ males would indicate that the Y and Y: pe^+ form the bivalent 48%[3] of the time and the frequency of X-Y and X-Y: pe^+ bivalents is 32 and

[3] The pairing percentages are estimated as follows: Let a, b, and c, (where $c = 1 - a - b$) equal the frequency of Y-Y: pe^+, X-Y, and X-Y: pe^+ bivalents, respectively. Now if the univalent passes at random at metaphase I, each bivalent will form four types of sperm with equal frequency as follows: Y-Y: pe^+ bivalents form X/Y: pe^+, Y, X/Y, and Y: pe^+ sperm; X-Y bivalents form X, Y/Y: pe^+, X/Y: pe^+, and Y sperm; X-Y: pe^+ bivalents form X, Y/Y: pe^+, X/Y, and Y: pe^+ sperm. If the frequency of the six sperm types given in table 2 are used, it can be seen that $a = 2(0.198) + 2(0.173) - b/2 - c/2$. Substituting $1 - a - b$ for c and solving, $a = 0.484$.

20%, respectively. Thus one would expect more homology between the Y and Y: pe^+ than between either X and Y, or between X and Y: pe^+. This reasonable conclusion is in agreement with KIKKAWA's interpretation of his data and, at least superficially, lends support to the hypothesis of a bivalent-univalent type of pairing. However, such a hypothesis becomes nonsensical if one considers the data from the X/Y: pe^+/Y: pe^+ males. On the stated hypothesis, one would expect that a bivalent would form more frequently between the two identical Y's than between the X and one of the Y's. However, the pairing relations estimated on this hypothesis indicate that a bivalent is practically never formed between the Y's (less than 5% pairing in the X/Y: pe^+/Y: pe^+ males). These data clearly show that the previously assumed picture of pairing is erroneous.

The alternative interpretation of the pairing relations is that the chromosome associations are multivalent and that the particular association of chromosomes involved in the multivalent determines the direction of disjunction. Cytological verification of this alternative was sought in a study of the late prophase and metaphase configurations seen in primary spermatocytes found in the testes of newly emerged males. Photomicrographs of the associations typically found at metaphase I in X/Y/Y: pe^+, X/Y: pe^+/Y: pe^+, and X/Y/Y: pe^+/Y: pe^+ males are shown in figure 1. Although many metaphase preparations were obtained and studied, in only one cell (from a X/Y/Y: pe^+ male) could a configuration possibly be interpreted as a bivalent and a univalent. All the associations were multivalent even in the X/Y/Y: pe^+/Y: pe^+ males where one might expect the formation of two bivalents. With the fixation and staining methods employed (some testes were dissected out and fixed in methyl alcohol-acetic acid mixtures and then stained and squashed in aceto-orcein; others were dissected out in saline and stained and squashed in aceto-orcein), it was impossible to distinguish which of the chromosomes of the multivalent was the X, the Y, or the Y: pe^+. Thus we were unable to get direct cytological confirmation of

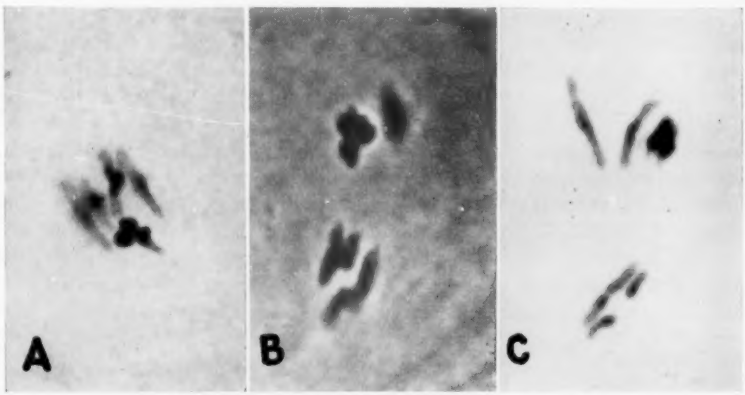

FIGURE 1.—Spermatocytes at metaphase I showing multivalent association. A. Trivalent in X/Y/Y: pe^+ male. B. Trivalent in X/Y: pe^+/Y: pe^+ male. C. Tetravalent in X/Y/Y: pe^+/Y: pe^+ male.

the segregation patterns obtained from genetic analysis. Further refinements of fixation might, however, allow such an analysis. It should be recalled that COOPER (1949) did check cytologically the types of segregation in X/Y/Y and X/Y/Y/Y males of *D. melanogaster*. Of 10 cells in the former males scored in anaphase I, seven were of the XY-Y type and three were X-YY, in good agreement with the 2:1 ratio of these types that would be expected on random segregation, thus supporting the genetic data. Sixty-seven anaphases were scored in X/Y/Y/Y males giving 76% XY-YY, 12% X-YYY, and 12% Y-XYY segregations. These results indicate another difference between *D. virilis* and *D. melanogaster* since only 3% of the segregations were of the 3-1 type in the former species. One wonders if this could be a reflection of the presumed more intimate chromosome pairing in *D. virilis*, since the total length of the crossover map in *D. virilis* is about three times that of *D. melanogaster*.

These findings raise two fundamental questions concerning pairing and disjunction in male Drosophila: (1) Why is multivalent association the rule, rather than the exception, when more than two chromosomes share homologies? Specifically, why do the sex chromosomes in X/Y/Y: pe^+/Y: pe^+ males not form two bivalents, one between the identical Y: pe^+ chromosomes and one between the X and Y? (2) What is the relation, if any, between the pairing forces in the multivalent and the direction in which the segregation is oriented? Consideration of the first question leads to the conclusion that the number or structural arrangement of the pairing sites in the X and Y chromosomes must differ in such a manner that more of the total pairing sites are satisfied with the formation of a tetravalent than with the formation of two bivalents. This then implies that at least some of the pairing sites that are not paired

TABLE 2

Disjunction in sex chromosome multivalents of Drosophila virilis

Type of male	Frequency of disjunctional types from multivalents							
	←→		←→		←→		←→	
X/Y/Y*	X		X		Y_a			
	Y_a	Y_b	Y_b		Y_a	Y_b	X	
		40%			40%		20%	
X/Y/Y:pe^+	X		X		Y			
	Y:pe^+	Y	Y		Y:pe^+	Y:pe^+	X	
		40%			34%		26%	
X/Y:pe^+/Y:pe^+	Y:pe^+_a		Y:pe^+_b		Y:pe^+_a			
	X	Y:pe^+_b	X		Y:pe^+_a	Y:pe^+_b	X	
		24%			24%		52%	
X/Y/Y:pe^+/Y:pe^+	X	Y	X		Y	X	Y:pe^+_a	
	Y:pe^+_a	Y:pe^+_b	Y:pe^+_b		Y:pe^+_a	Y	Y:pe^+_b	
		34%			34%		30%	

* Data from KIKKAWA (1935).

in an X-Y bivalent are able to pair with sites on the Y: pe^+ chromosomes. It is not difficult to devise formal schemes of pairing sites that would fulfill these qualifications and still be reasonable when the origin of the three types of chromosomes is considered.

The real difficulty becomes evident when one attempts to answer the second question. Any model of pairing relations in the multivalents must be compatible with the type and frequency of disjunction, as set forth in table 2, that takes place in the four kinds of males. Our data on X/Y/Y: pe^+ males do not indicate any difference between segregation in them and in the X/Y/Y males that KIKKAWA studied; however, in trivalents with two Y: pe^+ chromosomes, the disjunction is entirely different. In the first two types of trivalents listed, the Y chromosomes disjoin 75 to 80% of the time, whereas in the last type of trivalent, where identical marked Y's are present, they disjoin with a frequency of only 48%. It has usually been assumed that there is a positive correlation between the extent of homology of two chromosomes and the probability that they will disjoin, but the reverse seems to be true in these trivalents. Another way of looking at the same data would be to assume that the reason the X disjoins from the two normal Y's or from the normal Y and one Y: pe^+ more frequently than it disjoins from the two Y: pe^+'s is that, in the former cases, there are enough pairing sites on the X to satisfy most of the sites on the other two Y's. However, in a trivalent with two Y: pe^+'s, the added chromocentric region of chromosome 5 makes more pairing sites available than the X can satisfy. Such a scheme would be applicable whether there was two-by-two pairing at a given site or multiple pairing at a locus as the cytological pictures of COOPER (1949) would suggest. The apparently random 2-2 disjunction from the tetravalent must be added to this already confused picture.

Our inability to arrive at any definite model to explain the segregation results stems from our scant knowledge of multivalent association; it is difficult to place sufficient restrictions on possible events that could take place. Therefore, there is no method of determining the validity of a number of models that can be proposed. For example, we have no idea as to how the following factors influence the disjunctional pattern: orientation of the multivalent on the spindle, multiple pairing at a given site, the pairing configuration of the multivalent, and the manner in which the initial separation of chromosomes takes place. Progress on the problem of disjunction in sex chromosome multivalents will be made when a combined genetic and cytological study not only with marked sex chromosomes but also with those deficient for known regions that affect pairing is feasible. The success of such a study would depend on knowledge of the role that individual regions of the Y chromosome and chromocentral regions of the X play in disjunction. As COOPER (1956) has emphasized, a proper understanding of the part that the Y chromosome plays in various genetic effects awaits the adequate characterization of the functional properties of regions along its length.

SUMMARY

A study was made of chromosome segregation in *Drosophila virilis* males with extra Y chromosomes. Since the data from males with two Y chromosomes indicate

preferential segregation and are not compatible with the hypothesis that a bivalent and a univalent are formed at metaphase I, it was inferred that the association is multivalent. This inference was verified by a cytological study of primary spermatocytes in young imagoes. Sex chromosome trivalents and tetravalents are invariably observed in males with two or three Y chromosomes, respectively.

The segregation data indicate that the pattern of disjunction from trivalents is a function of the particular Y chromosomes involved. In X/Y/Y males with two normal Y's or with one normal and one marked Y, the Y's disjoin almost twice as frequently as they do from a trivalent with two identical marked Y's. Segregation from the tetravalents is almost entirely two-by-two with no evidence of preference for any of the three types of disjunction.

The inadequacy of our knowledge of the factors involved in multivalent association makes any interpretation of the segregation data tenuous.

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GENETIC INVESTIGATIONS OF THE WHORLED MUTANT IN GOSSYPIUM HERBACEUM[1]

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A WHORLED mutant in *G. herbaceum* has been reported from the Agricultural Research Station, Surat, Bombay State, India, by CHAVDA and PATEL (1954). It had some phenotypic resemblance to the cluster-short branch mutant reported previously (PATEL, MUNSHI, and PATEL, 1947) the important common characteristic being short one-noded fruiting branches terminating in more than one flower bud. While the cluster mutant has under-sized leaves, buds, flowers, peduncles and bears bolls mostly singly, the whorled mutant has normal sized organs except slightly longer peduncles and bolls in clusters. In addition, the whorled mutant has the following characteristics: (1) It has more than one monopodial branch at most nodes of the primaries, particularly those towards the terminal side, irrespective of whether they are monopodial or sympodial. The secondary monopodia, in turn, give rise to tertiaries which are one or two in the lower region, and 2 to 3 in the terminal region of the main stem giving the plant a whorled appearance. (2) Its leaves are thick and mostly 3-lobed instead of 5-lobed (palmate), as found in a normal herbaceum plant. (3) The bolls are narrow and elongated. Whorled was crossed with

Parental type	Phenotype	Genotype
1. 8–1 red herbaceum	Red (entire plant)	R_2/R_2
	3–4 loculed (mainly 3 loculed and rarely 4 loculed, which is normal in *G. herbaceum*)	Loc^{3-4}/Loc^{3-4}
2. Quintuple recessive	Cluster-short-branch[4]	cl_2^{BH}/cl_2^{BH}
	Ghost spot	R_2^{OS}/R_2^{OS}
	4–5 loculed (mainly 4 loculed and rarely 5 loculed)	loc^{4-5}/loc^{4-5}
	White flower	Ya/Ya, Yb^p/Yb^p
	Lintless fuzzy	lib/lib
3. Triple recessive	Ghost spot	R_2^{OS}/R_2^{OS}
	4–5 loculed	loc^{4-5}/loc^{4-5}
	White flower	Ya/Ya, Yb^p/Yb^p
4. Whorled	Whorled	cl_2^{WA}/cl_2^{WA}
	2–3 loculed (i.e., mainly 2 loculed and rarely 3 loculed	loc^{2-3}/loc^{2-3}

[1] The paper was read with only F_1 and F_2 data at the 42nd Indian Science Congress, Baroda. It is now finalised with the inclusion of F_3 data.

[2] Formerly at the Agricultural Research Station, Surat.

[3] Formerly at the Agricultural Research Station, Surat.

[4] This description is adopted as the type has the character expression of both the mutants described by HARLAND (1939).

cluster-short branch, firstly to determine the identity or otherwise of the whorled gene with that for cluster, and secondly to investigate its relationships with other mutant genes. The mutant plant was crossed with three herbaceum types, viz., 8–1 red, quintuple recessive, and triple recessive, developed at the Station. The special phenotypic characters and genetic constitution of the parental types involved in the crosses are mentioned against them below. Provisional gene symbols have been adopted for characters for which they have not so far been assigned.

The crosses were effected in 1951–52 and F_1, F_2 and F_3 generations were raised in 1952–53, 1953–54 and 1954–55 seasons at the Agricultural Research Station, Surat.

EXPERIMENTAL RESULTS

F_1 characters

The phenotypic expressions of the F_1 characters were as would be expected according to the usual allelic relationships except in the case of the following two characters:

Parental characters	F_1 characters
Cluster-short branch ♀ and whorled ♂ (and also reciprocal)	Normal
4–5 loculed ♀ and 2–3 loculed ♂ both individually recessive to 3–4 loculed	3–4 or 3–2 loculed

From the F_1 characters it was evident, firstly, that the cluster-short branch and whorled were mutants at different loci and that the two boll types, 4–5 loculed and 2–3 loculed which were individually recessive to 3–4 loculed (normal in *G. herbaceum*) were complementary in phenotypic expression.

F_2 segregations

(1) Cross 8–1 red × whorled mutant

The segregation data for the combination of characters plant color and branching habit are presented in table 1a and that for branching habit and locule type in table 1b.

The segregation data for the combination of characters plant color and locule type (mainly 3 loculed or mainly 2 loculed)[5] were identical with the data presented in table 1a.

The joint segregation for the two characters, red/green and normal/whorled (table 1a) indicates a linkage of 11.6 R.P. (recombination percentage). This is in fair agreement with 13.9 R.P. found by SILOW (1946) in the $R_2 - Cl_2$ chromosome of the amphidiploid hirsutum species. The small difference may probably be accounted for by the differentiation of the $R_2 - Cl_2$ chromosome of the A genome in the amphidiploid species and, to some extent, by the environmental differences.

Though the complete absence of recombinants between whorled and boll loculi characters in as many as 436 F_2 segregants (table 1b) should give the impression of an identical main locus for the two, as will be seen later, F_3 segregation showed their loci to be very closely linked. Further, the facts that (1) the 2–3 and 4–5 loculi types are complementary in expression (2) the 3–4 loculed types breed true (3) the crossover

[5] Mainly 3 loculed included plants with 3, 3-2, 3-4 and 3-4-2 loculed bolls. Mainly 2 loculed included plants with 2 and 2-3 loculed bolls.

TABLE 1a

The results of combined segregation of plant colour and the mutant whorled branching habit in the F_2 of the cross 8–1 red × whorled mutant

Red		Green		Total	x^2			P
Normal	Whorled	Normal	Whorled		Red/green	Normal/whorled	Linkage	
306	18	29	83	436	0.11	—	—	0.7 to 0.8
					—	0.78	—	0.3 to 0.5
					—	—	211.96	very low.

TABLE 1b

The results of segregation in the F_2 for the combination of characters whorled branching habit and the locule number (mainly 3 loculed or mainly 2 loculed) in the cross 8–1 red × whorled mutant

Normal		Whorled		Total	x^2			P
Mainly 3 loculed[5]	Mainly 2 loculed[5]	Mainly 3 loculed	Mainly 2 loculed		Normal/whorled	3 loc/2 loc	Linkage	
335	—	—	101	436	0.78	—	—	0.3 to 0.5
					—	0.78	—	0.3 to 0.5
					—	—	394.38	very low

types, e.g. (3 loculed, whorled) and (2 loculed, cluster-short branch), which have occurred in the remaining two crosses are very rare, indicate that the loci for 2–3 and 4–5 are closely linked. The character seems to be influenced by modifiers since, as previously stated, even among plants having predominantly 2 and 3 loculed bolls, variants, e.g. 2–3 and 3–2, 3–4, 3–4–2, were found.

(2 & 3) Crosses of whorled with quintuple and triple recessive types

In crosses of the whorled mutant with the quintuple and triple recessive types in both of which the recessive anthocyanin allele, ghost spot, was involved, it was found that the ghost spot segregates were viable on the whole to the extent of only about 67 percent, though the parental types containing the ghost spot gene were normal in viability. Similar deficiencies of ghost spot segregates were met with in large scale segregations, firstly in the cross between the triple recessive and Garo Hill by C. T. PATEL (unpublished) and, secondly, in the cross between the quintuple recessive and 2087-curled by the present authors. The observed joint segregation for the cluster-short branch and petal spot characters is given in table 2a. Correction for deficient ghost spot (zygotic) viability renders the data suitable for linkage estimation.

The joint segregation, whether as corrected or otherwise, is indicative of a recombination value which is almost equal to one of 30 percent found by G. B. PATEL *et al.* (1947) in a cross between cluster-short branch and ghost spot mutants, in which deficiencies of ghost spot segregates were not observed. It would appear, therefore, that the locus for anthocyanin is linked with the locus for whorled with 11.6 percent recombination and with the cluster-short branch locus with about 30 R.P.

The character combinations, viz., (1) spot-whorled and (2) spot-boll/loculi in both

TABLE 2a

The results of segregation for the combination of characters branching habit and petal spot in the F_2 of the cross quintuple recessive and whorled

spotted	Non-Cluster including whorled		Cluster-short branch (including cluster-whorled)		Total	χ^2			P
	Spotted	G. spot	Spotted	G. spot		Normal /*cl-sh br*	*Sp/G S*	Linkage	
Observed frequencies									
Whorled × q.r.	89	9	18	8	124				
Q.r. × whorled	19	6	2	6	33				
Total	108	15	20	14	157				
Observed frequencies corrected for 33% deficiency of ghost spot segregates									
Whorled × q.r.	89	13	18	12	132	0.36	—	—	0.5 to 0.7
						—	2.59	—	0.1 to 0.2
						—	—	9.1	0.001 to 0.01
Q.r. × whorled	19	9	2	9	39	0.21	—	—	0.5 to 0.7
						—	9.31	—	0.001 to 0.01
						—	—	12.79	0.001 to 0.01
Total	108	22	20	21	171	0.96	—	—	0.3 to 0.5
						—	0.002	—	0.9 to 0.95
						—	—	19.0	very low.

the crosses could not be utilised in view of the fact that in both these cases nil frequencies of segregates involving the ghost spot gene were encountered and were not amenable to correction for ghost spot deficiency.

The segregations for other combinations, viz., (1) cluster-whorled[6] and (2) cluster/whorled-boll loculi in only 'spotted' plants in the cross with q.r. are given in table 2b for what they are worth. On the basis of these data, the estimate of recombination between these two loci is 18.8 which is compatible with R.P.'s of 30% between cluster and the spot loci and 11.6% between whorled and spot loci, if both cluster and whorled are on the same side of the spot locus.

Cluster/whorled-boll loculi segregation in spotted plants.—The cluster-boll loculi segregation in the crosses with the quintuple recessive are given in table 2c. The frequency distribution of these characters appears to follow a 9:7 ratio. Assuming the estimate of q, the R.P. between whorled and boll-loculi number, to be 0.005 as reliably determined from undisturbed F_2 and F_3 segregations (vide table 4, page 921), the estimate of p from the above data would be about 0.25, a value fairly comparable

[6] The cluster-whorled segregate has the following characters of both of its parents and is, therefore, clearly distinguishable from them. Characters of cluster are reduced size of leaves, buds, flowers, peduncles and bolls. Characters of whorled are mostly 3-lobed thick leaves, bolls borne in clusters, and whorled appearance of the plant.

TABLE 2b

The results of segregation for the combination of the mutant characters cluster and whorled in spotted plants from the F_2 progeny of the quintuple recessive and whorled

	Normal	Cluster	Whorled	Cluster-whorled	Total
Whorled × q.r.	55	17	34	1	107
Q.r. × whorled	12	2	7	—	21
Total	67	19	41	1	128

TABLE 2c

The results of segregation for the combination of characters branching habit and the locule number of the boll in the F_2 of the cross whorled and quintuple recessive

	Normal		Cluster & whorled		Total
	Mostly 3 loculed	Other than 3 loculed	Mostly 3 loculed	Other than 3 loculed	
Whorled × q.r.	38	15	18	34	105
Q.R. × whorled	8	4	5	4	21
Total	46	19	23	38	126

with 18.8%. This may be considered to be satisfactory in view of the fact that the data are salvaged from disturbed segregations.

Segregations of flower colour and lintlessness in combination with cluster, whorled and boll loculi number in spotted plants are given in Appendix I. It is not thought worthwhile to discuss them here, firstly, because in each combination, the segregations are complicated on account of one of the characters being governed by linked complementary genes, and secondly, because the genes for flower colour and lintlessness have already been proved by other workers to be on other chromosomes. It may only be pointed out that the following joint segregations indicate independence of the characters considered together:

Cross	Joint segregation
Triple recessive × whorled	Whorled—flower colour
Q.r. × whorled and whorled × q.r.	Lintlessness—flower colour

In view of the disturbance caused in segregations by the ghost spot gene, the crosses of whorled with both the triple recessive and quintuple recessive were not continued in F_3. Only six backcrosses of which small numbers of seed were available were sown.

F_3 segregations[7]

Only the cross, 8–1 red × whorled was followed up in F_3 as the other two crosses involved the disturbing ghost spot gene. The season of 1954–55 having proved to be one of extraordinarily heavy rainfall at Surat and the plot which was available for

[7] For details see Appendix II.

TABLE 3a

The distribution of plant body colour, branching habit and locule number in the boll in F_3 families showing segregation for the three characters

Plant body colour			Branching habit			Boll loculi number		
Red	Green	Total	Normal	Whorled	Total	Mainly 3–4	Mainly 2–3	Total
971	307	1278	963	315	1278	970	308	1278
Probabilities:								
deviation from 3:1 ratio		0.3 to 0.5			0.7 to 0.8			0.3 to 0.5
heterogeneity (14 d.f.)		about 0.2			about 0.2			about 0.5

TABLE 3b

The results of combined segregations for plant colour, branching habit, and locule number in the boll in F_3 families of the cross 8–1 red × whorled

Red, normal	Red, whorled	Green, normal	Green, whorled	Total
900	71	63	244	1278
Normal 3–4 loculi	Normal 2–3 loculi	Whorled 3–4 loculi	Whorled 2–3 loculi	Total
962	1	8	307	1278
Red, 3–4 loculi	Red, 2–3 loculi	Green, 3–4 loculi	Green, 2–3 loculi	Total
899	72	71	236	1278

growing the F_3 cultures having thereby been waterlogged, the first sowing of selfed seed of 40 random, triple dominant F_2 plants of this cross and the six backcrosses of whorled × q.r. and t.r. proved to be a total failure. Sufficient selfed seed for the second sowing was available from only 31 random F_2 plants of this cross. Out of the 31 cultures raised, 12 proved to be true-breeding, 2 segregated for only plant colour, red/green, 2 for only two characters, viz., normal/whorled and 3–4/2–3 loculi and 15 for all the three. All joint segregations in all the 17 cultures in the last two categories were in coupling; but, in the 2 cultures which segregated for only two characters, the single factor segregation was very abnormal for both the characters, the χ^2 values for normal/whorled being 5.80 and 3.96 and those for 3–4/2–3 loculi being 3.71 and 4.93, respectively, for single degrees of freedom. Again, the two cultures are not homogeneous between themselves in regard to segregation for any of the two characters, the χ^2 values for heterogeneity being 9.72 for normal/whorled and 8.56 for 3–4/2–3 loculi each for one degree of freedom. Therefore, the data of only the 15 families segregating for all the characters, and as will be seen later are also homogeneous,were taken for estimation. The segregation data are given in tables 3a and b.

Two of the families viz., nos. 3023 and 3035 consisting of 14 and 74 plants, respectively, deviated from the 3:1 ratio with probabilities up to 0.02. Family 3023 deviated only in respect of the colour character and 3035 in all the three characters.

TABLE 4

The results of combined estimation based on data from F_2 and 15 F_3 families segregating in coupling

Character combinations	Recombination percentage	Probabilities at the estimated R.P.		
			Deviation	Heteogeneity (between)
Red/green and normal/whorled	11.3	F_2 & F_3	0.99	0.8 to 0.9
	±0.0082	F_3 families (14)	0.99	0.8
Normal/whorled and 3–4/2–3	0.53	F_2 & F_3	0.99	about 0.1
	±0.0055	F_3 families (14)	0.3 to 0.5	about 0.7
Red/green and 3–4/2–3 loculi	11.95	F_2 & F_3	0.99	0.8
	±0.0085	F_3 families (14)	0.9	0.5 to 0.7

TABLE 5

The observed and expected frequency of true breeding families and those segregating with regard to one, two, or three factors

	True-breeding	Segregating for all three factors				Segregating for only two factors		Segregating for one factor
		C C*	C R	R C	R R	C	R	
Observed	12	15	0	0	0	2	0	2
Expected	8.680	17.361	0.001	0.143	0	2.328	0.013	2.475

* C = coupling; R = repulsion.

It will be seen from the data presented in table 4 that the findings made from F_2 data are confirmed by the F_3 segregations in regard to the estimate of linkage between the anthocyanin and whorled characters. Whorled is found to be very closely linked with the boll loculi allele, 2–3 loculi, and not identical with it as should have been thought from the F_2 data. It would also appear that the whorled locus is situated between the loci for anthocyanin and boll loculi.

By KOSAMBI's formula, the R.P. in the segment between red/green and 3–4/2–3 loculi should be 11.80. The actual estimate of 11.95 is in fairly good agreement with this.

The expected distribution of the 31 families into true-breeding and various segregating classes on the basis of the estimates made from the F_2 and F_3 data together, is compared with the observed in table 5. The probabilities for 7 d.f. are 0.95 to 0.98 showing very good agreement. Thus, the segregations between and within families are homogeneous at the estimated R.P.'s for the chromosome segments under consideration.

It is not thought useful to discuss other character combinations as their segregations have been badly disturbed by the ghost spot gene.

DISCUSSION AND CONCLUSIONS

The genetic investigations of the whorled mutant in *G. herbaceum* had led to the finding that the genes, (1) cluster-short branch (cl_2^{BH}) and (2) whorled (c_2^{Wh}), which are phenotypically more or less alike and are complementary in expression, are

situated on either side of the anthocyanin locus of the R_2 chromosome. The recombination percentages between (1) cl_2^{BH} and R_2^{OS} and (2) cl^{Wh}_2 and R_2 have been found to be about 30.0% and 11.3%, respectively. These recombination percentages are more or less in agreement with those found by previous workers. Moreover, the main gene for 2–3 boll loculi number is found to be very closely linked (R.P. = 0.53) with the whorled gene on one side and also closely linked with and complementary to the gene for 4–5 loculed, though the recombination frequency between them could not be estimated on account of the deficient viability of ghost spot segregates. The loculi genes are subject to the influence of modifiers; but complications of the sort explained by SAYAR (1952) through the assumption of multigenes were not met with.

The two types of complementarity of loci situated on one and the same chromosome as exhibited by the two characters viz., branching habit and boll loculi number, more particularly the former, are of uncommon occurrence. While the former may probably be the result of an inversion or a translocation, the latter belongs to the category of complex loci (DUNN 1954), the locus for boll loculi character comprising the two complementary pseudoalleles, 2–3 and 4–5.

The occurrence of the ghost spot gene in segregates of the crosses of whorled with the quintuple recessive and the triple recessive types disturbed the segregations since, as stated above, the ghost spot phenotypes were deficient in viability to the extent of about 33 percent in both the crosses. For this reason, only the cross, (8–1 red × whorled) proved to be fully informative. The amount of deficiency in viability of the ghost spot segregates agrees with what has been found in the cross between the same triple recessive and Garo-Hill types and that between the q.r. and 2087 curled. This deficiency in viability does not appear universal since it was not observed in the cross between cluster-short branch and ghost spot types. There is, therefore, reason to infer that ghost spot is a character involving linked balanced lethals. It is rather striking that not a single whorled plant was observed amongst a total of 41 ghost spot segregates.

STEPHENS (1951) has illustrated his hypothesis of the evolution of the specificity of genes through the relationship of the cluster (cl_1) and short branch (cl_2) genes located respectively in the $R_2 - Cl_2$ linkage group of the D and A genomes constituting the amphidiploid species. The details of the case as given by him are as follows: (1) The normal allele at one locus cannot mask the mutant allele at the other locus in either case; (2) The two mutants behave as complementaries and (3) The cl_1Cl_2/Cl_2Cl_2 hybrid obtained by crossing cluster with an autotetraploid A type is phenotypically normal.

From the above relationships he infers, "The Cl_2 normal allele carried by the A type is able to mask the mutant allele, cl_1, whereas the homologous normal allele in the amphidiploid cannot. At present, though, the possibility that this apparent difference in masking ability is a question of Cl_2 dosage cannot be excluded." The finding in the present investigation of two complementary loci with more or less similar phenotypic expression in the $R_2 - Cl_2$ linkage group makes it possible to explain in a different way, the relationships reported between Cl_1 and Cl_2 loci, if it be that in the amphidiploid the two complementary loci, cl^{BH} and cl^{Wh} are present

singly in the D and A genomes without having their strict counterparts in the A and D genomes respectively, as is actually the case with alleles of the R_1 and R_2 loci. On this basis, the cluster and short branch types found in the amphidiploid will be of the following genotypes:

Cluster	cl_1^{BH}	R_1	Cl^{Wh}	R_2
	cl_1^{BH}	R_1	Cl_2^{Wh}	R_2
Short branch	Cl_1^{BH}	R_1	cl_2^{Wh}	R_2
	Cl_1^{BH}	R_1	cl_2^{Wh}	R_2

A cross between the two mutants will evidently be normal. Further, a cross between the cluster mutant and an autotetraploid produced from the normal A type diploid which (latter) will have both Cl_2^{BH} and Cl_2^{Wh} dominant genes in the R_2 chromosome will also produce a normal hybrid.

The present investigations of crosses involving the whorled mutant as one of the parents indicate that the linear arrangement in terms of recombination percentages of genes governing the characters (1) branching habit and (2) boll loculi number on the R_2 chromosome of *G. herbaceum* is as shown in figure 1.

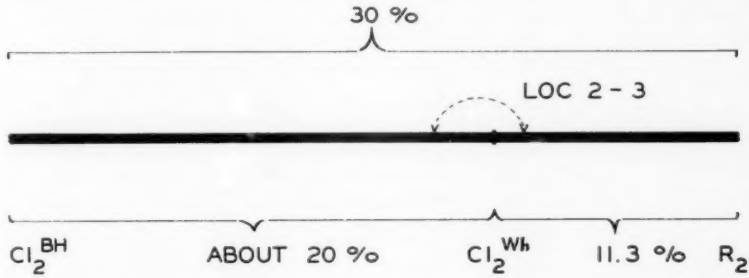

FIGURE 1.—Linkage of genes governing branching habit and boll loculi number on the R_2 chromosome.

SUMMARY

The paper relates to the genetic investigations of the whorled mutant in *G. herbaceum* carried out at Surat, India, by crossing it with three herbaceum types developed at the Station, viz., (1) 8–1 red (2) ghost spot, white flower, 4–5 loculed triple recessive and (3) cluster-short branch, ghost spot, white flower, 4–5 loculed, lintless quintuple recessive. Only the first cross proved to be fully informative, the latter two having suffered from deficiency of about 33 percent viability in ghost spot segregates.

The investigations revealed that: (1) Cluster-short branch and whorled were linked complementary loci situated at about 30 and 11.3 R.P.'s, respectively, on same side of the anthocyanin locus. (2) The boll loculi character was governed by a pair of complementary pseudoalleles, $loc^{2\text{-}3}$ and $loc^{4\text{-}5}$ of which the former is very closely linked at 0.53 R.P. with the whorled locus. The R.P. between the pseudoalleles which is small remains undetermined. (3) The linear arrangement of genes

governing the characters under study, viz., anthocyanin, branching habit and boll loculi number has been indicated. (4) A hypothesis alternative to the one of evolution of specificity of genes proposed by STEPHENS (1951) is offered to explain the relationship of the cluster (cl_1) and short branch (cl_2) genes located respectively in the $R_2 - cl_2$ linkage group of the D and A genomes constituting the amphidiploid species.

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APPENDIX I

F_2 segregations in 'spotted' plants

TABLE a

Parents: ♀ Normal (t.r.), white flowered.
♂ Whorled, yellow flowered.
F_1: Normal, yellow flowered, 3–2 loculed.

F_2 segregation:

Normal		Whorled		Total
Yellow flowered	White flowered	Yellow flowered	White flowered	
38	13	15	2	68

TABLE b

Parents: ♀ Normal (t.r.), white flowered, 4–5 loculed.
♂ Whorled, yellow flowered, 2–3 loculed.
F_1: Normal, yellow flowered, 3–2 loculed.

F_2 segregation:

	No. of boll loculi								
Flower colour	2	2–3	3	3–2	3–4	4–3	4–5	5–4	Total
Yellow	8	6	4	25	4	2	4	—	53
White	—	1	—	9	2	—	3	—	15
Total	8	7	4	34	6	2	7	—	68

TABLE c

Parents: ♀ Whorled, yellow flowered, 2–3 loculed.
♂ Cluster (q.r.), white flowered, 4–5 loculed.
F_1: Normal, yellow flowered, 3–2 loculed.

F_2 segregation:

Flower colour	No. of boll loculi								
	2	2–3	3	3–2	3–4	4–3	4–5	5–4	Total
Yellow	11	11	5	30	6	1	13	1	78
White	4	4	1	8	6	—	3	—	26
Total	15	15	6	38	12	1	16	1	104

TABLE d

Parents: ♀ Cluster (q.r.), white flowered, 4–5 loculed.
♂ Whorled, yellow flowered, 2–3 loculed.
F_1: Normal, yellow flowered, 3–2 loculed.

F_2 segregation:

Flower colour	No. of boll loculi								
	2	2–3	3	3–2	3–4	4–3	4–5	5–4	Total
Yellow	2	1	2	8	1	1	3	—	18
White	—	—	—	1	1	—	1	—	3
Total	2	1	2	9	2	1	4	—	21

TABLE e

Parents ♀ Whorled, 2–3 loculed, linted fuzzy.
♂ Cluster (q.r.), 4–5 loculed, lintless fuzzy.
F_1: Normal, 3–2 loculed, linted fuzzy.

F_2 segregation:

Lint characters	No. of boll loculi								
	2	2–3	3	3–2	3–4	4–3	4–5	5–4	Total
Linted fuzzy	11	9	5	30	8	1	14	—	78
Lintless fuzzy	5	6	—	9	2	—	3	1	26
Total	16	15	5	39	10	1	17	1	104

TABLE f

Parents: ♀ Cluster (q.r.), 4–5 loculed, lintless fuzzy.
♂ Whorled, 2–3 loculed, linted fuzzy.
F_1: Normal, 3–2 loculed, linted fuzzy.

F_2 segregation:

Lint characters	No. of boll loculi								
	2	2–3	3	3–2	3–4	4–3	4–5	5–4	Total
Linted fuzzy	2	—	2	7	1	—	3	—	15
Lintless fuzzy	—	1	—	2	1	1	1	—	6
Total	2	1	2	9	2	1	4	—	21

TABLE g

Parents: ♀ Whorled, linted fuzzy.
♂ Cluster (q.r.), lintless fuzzy.
F_1: Normal, linted fuzzy.

F_2 segregation:

Normal		Whorled		Cluster		Cluster-whorled		Total
LTF*	LLF	LTF	LLF	LTF	LLF	LTF	LLF	
46	7	23	11	10	6	—	1	104

* LTF = linted fuzzy; LLF = lintless fuzzy.

TABLE h

Parents: ♀ Cluster (q.r.), lintless fuzzy.
♂ Whorled, linted fuzzy.
F_1: Normal, linted fuzzy.

F_2 segregation:

Normal		Whorled		Cluster		Cluster-Whorled		Total
LTF	LLF	LTF	LLF	LTF	LLF	LTF	LLF	
9	3	5	2	1	1	—	—	21

TABLE i

Parents: ♀ Whorled, yellow flowered.
♂ Cluster (q.r.), white flowered.
F_1: Normal, yellow flowered.

F_2 segregation:

Normal		Whorled		Cluster		Cluster-whorled		Total
Yellow	White	Yellow	White	Yellow	White	Yellow	White	
44	11	23	11	13	4	1	—	107

TABLE j

Parents: ♀ Cluster (q.r.), white flowered.
♂ Whorled, yellow flowered.
F_1: Normal, yellow flowered.

F_2 segregation:

Normal		Whorled		Cluster		Cluster-whorled		Total
Yellow	White	Yellow	White	Yellow	White	Yellow	White	
10	2	6	1	2	—	—	—	21

TABLE k

Parents: ♀ Whorled, linted fuzzy, yellow flowered.
♂ Cluster (q.r.), lintless fuzzy, white flowered.
F_1: Normal, linted fuzzy, yellow flowered.

F_2 segregation:

Linted fuzzy		Lintless fuzzy		Total
Yellow flower	White flower	Yellow flower	White flower	
56	22	21	4	103

TABLE l

Parents: ♀ Cluster (q.r.), lintless fuzzy, white flowered.
♂ Whorled, linted fuzzy, yellow flowered.
F_1: Normal, linted fuzzy, yellow flowered.

F_2 segregation:

Linted fuzzy		Lintless fuzzy		Total
Yellow flower	White flower	Yellow flower	White flower	
13	2	5	1	21

APPENDIX II

F_3 segregations for red/green—normal/whorled

S. no.	Culture no.	Phenotype of F_2 parent	Frequencies				Total
			R, N	R, W	G, N	G, W	
1	2636	R, N, 3-2	104	9	9	18	140
2	2647	R, N, 3-2	93	6	6	28	133
3	2796	R, N, 3-2	71	6	4	25	106
4	2826	R, N, 3-2	92	10	11	31	144
5	2678	R, N, 3	45	4	6	11	66
6	2862	R, N, 3-2-4	51	6	3	14	74
7	2712	R, N, 3-2	68	6	4	16	94
8	2967	R, N, 3-2	34	—	2	8	44
9	2737	R, N, 3-2	47	3	3	18	71
10	3007	R, N, 3-2	43	5	2	10	60
11	3023	R, N, 3-4-2	7	—	1	6	14
12	2950	R, N, 3	50	5	2	21	78
13	3001	R, N, 3-2	66	4	5	17	92
14	3011	R, N, 3	68	5	3	12	88
15	3035	R, N, 3-2	61	2	2	9	74
Total			900	71	63	244	1278

F_3 segregations for red/green—$3^{1oe}/2^{1oe}$

S. no.	Culture no.	Phenotype of F_2 parent	Frequencies				Total
			R, 3	R, 2	G, 3	G, 2	
1	2636	R, N, 3-2	103	10	9	18	140
2	2647	R, N, 3-2	93	6	7	27	133
3	2796	R, N, 3-2	71	6	6	23	106
4	2826	R, N, 3-2	92	10	13	29	144
5	2678	R, N, 3	45	4	6	11	66
6	2862	R, N, 3-2-4	51	6	4	13	74
7	2712	R, N, 3-2	68	6	5	15	94
8	2967	R, N, 3-2	34	—	2	8	44
9	2737	R, N, 3-2	47	3	3	18	71
10	3007	R, N, 3-2	43	5	3	9	60
11	3023	R, N, 3-4-2	7	—	1	6	14
12	2950	R, N, 3	50	5	2	21	78
13	3001	R, N, 3-2	66	4	5	17	92
14	3011	R, N, 3	68	5	3	12	88
15	3035	R, N, 3-2	61	2	2	9	74
Total			899	72	71	236	1278

F_3 segregations for normal/whorled—$3^{loc}/2^{loc}$

S. no.	Culture no.	Phenotype of F_2 parent	Frequencies				Total
			N, 3	N, 2	W, 3	W, 2	
1	2636	R, N, 3-2	112	1	—	27	140
2	2647	R, N, 3-2	99	—	1	33	133
3	2796	R, N, 3-2	75	—	2	29	106
4	2826	R, N, 3-2	103	—	2	39	144
5	2678	R, N, 3	51	—	—	15	66
6	2862	R, N, 3-2-4	54	—	1	19	74
7	2712	R, N, 3-2	72	—	1	21	94
8	2967	R, N, 3-2	36	—	—	8	44
9	2737	R, N, 3-2	50	—	—	21	71
10	3007	R, N, 3-2	45	—	1	14	60
11	3023	R, N, 3-4-2	8	—	—	6	14
12	2950	R, N, 3	52	—	—	26	78
13	3001	R, N, 3-2	71	—	—	21	92
14	3011	R, N, 3	71	—	—	17	88
15	3035	R, N, 3-2	63	—	—	11	74
Total			962	1	8	307	1278
16	3008	R, N, 3	57	2	4	29	92
17	2669	R, N, 3-2	85	1	2	15	103

MUTANTS AND LINKAGE IN MORMONIELLA

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THE research of various workers has resulted in the accumulation of a large number of mutant stocks of *Mormoniella vitripennis* (WALKER). Some of these mutants have been described by WHITING (1951); more recently, many others have arisen spontaneously or as the result of radiation studies conducted by KAYHART (1956) and SAUL (1955).

Many of the mutants are at the complex locus which WHITING (1951) has named the *R* locus, but mutations at other loci have also been found. Because of the increasing use of Mormoniella as research material, it is desirable to describe these mutants, and to summarize their linkage relationships. It would further be desirable to have available a linkage map corresponding to the five chromosomes of Mormoniella; this study is a contribution toward that goal.

MATERIALS AND METHODS

Mormoniella is a chalcidoid Hymenopteron parasitic on the pupae of various muscoid Diptera. Males are haploid and arise from unfertilized eggs; diploid females arise from fertilized eggs. Unmated females thus produce only male progeny; the ratio of phenotypes among these approximates the segregation of genes among the gametes of the parent females. The blowfly *Sarcophaga bullata* Parker is used as the host in the experiments to be reported here. Details of the life cycle of Mormoniella are described by SAUL (1955).

The wild type wasps have reddish-brown eyes and iridescent bronze-colored bodies. Females have darker antennae and body color than have the males. The wings of the females are of normal size; the males are brachypterous.

Most of the mutations obtained to this time affect either eye color or body color. The mutant eye colors vary from black through deep red, orange-red, orange and light peach, to oyster-white. In general, double mutants are in color as light as or lighter than either single mutant type. Body colors include purple, various blue-green shades, and deep blue. A lavender body color mutant was obtained, but it was lost before it could be tested for linkage. Mutants are kept as pure stocks unless they are female-sterile, in which case heterozygous females are crossed with mutant males. All of the mutants are recessive to wild type.

In this study, standard conventions are followed in designating the mutants and previously published names of mutants are retained. Eye color mutants for which a name has not been published are named, when possible, by reference to colored plates in *A Dictionary of Color* (MAERZ and PAUL 1950). The number of the plate which corresponds most closely to the eye color is used in the description of the mutant, and where possible the name attached to the plate is used for the mutant. Other mutants, such as body color mutants, which can not be named by this method, are named arbitrarily.

The apparatus used for comparing eye colors with the Maerz-Paul color charts was designed by DR. SARAH FLEMISTER. The wasp is observed by reflected light under the low-power objective and 10× ocular of a compound microscope. It is placed on its side, with the surface of the eye in full view. The color charts are covered with a piece of black cardboard which has an opening in the center; this opening permits comparison of the eye color with one tab at a time. A spotlight illuminates the eye and a second spotlight illuminates the color chart, which can be observed by shifting one's view only slightly from the microscope. All observations were made on freshly eclosed males.

Linkage tests were made by two factor crosses. In general, five males of one mutant type were crossed with five females homozygous for a second mutant gene. Five F_1 females from the cross were set unmated and the F_2 progeny, all male, were counted. Linkage, when present, was shown by reductions in the numbers of wasps in the recombinant classes (wild type and, in the absence of epistasis, double mutants).

If both mutants to be crossed were female sterile, P_1 females heterozygous for one of the mutant types were used. Each F_1 female was set individually and only those F_2 cultures which contained both mutant types were used for linkage counts. These cultures would be expected to include about half of the total number set.

If a cross indicated that two traits were linked, then, when possible, five males of the double-mutant type were crossed with five homozygous wild type females and the cross was carried to the F_2 generation as described above. In these cases linkage was thus estimated from crosses in the coupling and in the repulsion phases.

When possible, the calculation of linkage followed the geometric mean averaging system given by FISHER in *The Design of Experiments* (1949). The gametic ratio r/s is given by:

$$\sqrt{\frac{a_1 a_2}{b_1 b_2}}.$$

In this formula, a_1 and a_2 are the total recombinants from the coupling and repulsion experiments respectively; b_1 and b_2 are the total parentals from these crosses. This method compensates for differences in viability of the classes, but does not compensate for linked lethals.

In the case of epistasis, linkage was estimated from the cross in repulsion phase by dividing the number of wild type wasps (m) by the total number of wasps in the wild type class and the class (n) showing the hypostatic trait. The formula is thus $m/(m + n)$. The formula $n/(m + n)$ gives an estimate of linkage from the cross in the coupling phase. Since the double mutants could not be selected with certainty, several crosses were made for the coupling experiment. Each mating involved a single male from the class showing the epistatic trait and a wild type female; only F_2 cultures which included wasps showing the hypostatic trait (and which thus were sired by double-mutant males) were used for linkage counts. The geometric mean averaging system employed for more precise measurement of linkage can, in this case, use only one class of parentals and one of recombinants from each cross, since the other classes are identical due to the epistasis. In the general formula given above,

a_1, a_2, b_1, and b_2 represent only the distinguishable parental and recombinant classes; the method thus becomes the same as that proposed by MULLER (1916).

In F_2 cultures from some crosses, only wild type and one class including both the single-mutant and the double-mutant types could be distinguished. In these cases, linkage was measured by doubling the number in the wild type class and dividing it by the total count in the culture.

MUTANTS

The following list is a brief summary of information about 22 mutant types of Mormoniella. Apparent isoalleles are not included, nor are mutations that were lost before they could be tested and characterized adequately. Important information about the factors scarlet-DR, tomato, garnet, and purple has been reported by WHITING (1951); these factors are therefore listed here with only the Maerz-Paul references (where applicable) and literature citations. Of the numerous alleles at the complex eye color locus *R*, only those used in linkage tests are listed. The following information is given for all mutants except those mentioned above: symbol, name, investigator who found the mutant, date found, inducing agent (if any), description and important characteristics, and Maerz-Paul reference (if applicable). The Maerz-Paul reference includes the plate and tab numbers.

bk: black. GROESBECK 1952. Induced by X-rays. Eyes black, body slightly greener than wild type. Interacts with dark red eye colors to give lavender or light peach and with scarlets and lighter colors to give white. 48:L-12.

bl: blue. KAYHART 1953. Spontaneous in wild type stock. Body dark blue. Female sterile. Viability considerably lower than wild type.

ga: garnet. 3:L-11. (WHITING 1951).

$ga^{st\text{-}b}$: garnet-scarlet-b. KAYHART 1953. Induced by fast neutrons. Eyes orange-red, phenotype identical to $R^{st\text{-}DR}$ 2:L-11.

gl: glass. MILROOD 1952. Spontaneous. Eyes narrow, facets poorly differentiated and reduced in number. Female almost sterile. Viability somewhat lower than wild type.

gr: green. KAYHART 1953. Induced by slow neutrons. Body grass-green. Female sterile, viability somewhat lower than wild type.

gb: green-blue. WHITING 1952. Spontaneous from wild type. Body greenish-blue to deep blue. Female sterile.

or: orange. SAUL 1952. Induced by X-rays. Eye color dull orange. 2:D-12.

pl: pellucid. WHITING 1952. Spontaneous from +/*ti*-277. Eye color oyster-white, phenotype identical to $R^{oy\text{-}DR}$. Epistatic to all other eye colors. 4:A-7.

pu: purple. (WHITING 1951).

rh: reddish. KAYHART 1953. Induced by slow neutrons. Eye color dark rust-red. 6:I-11.

$R^{st\text{-}A\text{-}DR}$: Scarlet-DR.2:L-11. (WHITING 1951).

$R^{st\text{-}426}$: scarlet 426. GROESBECK 1952. Induced by X-rays. Phenotypically identical to scarlet-DR.2:L-11.

st-c: scarlet-c. WHITING 1953. Spontaneous from wild type. Phenotypically identical to scarlet-DR.2:L-11.

TABLE 1

Independent assortment of traits in Mormoniella, including data from representative two-factor crosses. Genes are listed alphabetically; crosses of each gene, in order, with succeeding genes are grouped together. A dash in the list of F_2 classes from a cross indicates that the class is included with another class identical or epistatic to it. The symbol a/b *means that the F_1 female is heterozygous for factors crossed in repulsion phase;* ab/+ *indicates factors crossed in coupling*

F_1 female from cross $a \times b$ or $ab \times +$	F_2 males			
	+	*a*	*b*	*ab* (with phenotype)
bk/bl	72	87	58	56
bk/ga	41	39	29	13 pale red
bk/gl	98	99	83	66
bk/gb	40	61	39	57
bk/or	76	79	79	95 oyster
bk/pl	49	53	119	—
bk/rd	71	75	77	62 deep gentian
bk/R$^{st\text{-}DR}$	69	61	58	56 oyster
bk/st-C	54	60	62	57 oyster
bk/st-d	68	118	72	81 oyster
bk/tl	114	103	107	124 pale red
bk/to^{mz}	72	63	74	55 dark red
bk/+	71	84		
bl/ga$^{st\text{-}b}$	95	93	73	85
bl/gl	65	56	65	61
bl/pl	80	50	59	65
bl/rd	38	32	37	30
bl/st-c	60	66	81	70
bl/st-d	56	56	45	46
bl/+	118	90		
ga/gl	103	78	104	63
ga/gb	84	80	85	99
ga/or	99	103	78	79 oyster
ga/pl	40	38	76	—
ga/rd	109	95	97	90 scarlet
ga/R$^{st\text{-}DR}$	96	94	212	—
ga/st-c	96	95	110	87 oyster
ga/st-d	69	80	97	73 oyster
ga/to^{mz}	100	87	74	72 scarlet
ga/+	157	138		
gl/gb	44	44	39	32
gl/pl	51	53	55	55
gl/rd	35	31	31	24
gl/st-c	78	66	97	60
gl/st-d	77	73	73	51
gl/tl	54	78	54	61
gl/to^{mz}	60	62	59	47
gl/+	133	111		
gb/or	75	66	58	78
gb/pl	62	59	57	57
gb/R$^{st\text{-}426}$	100	92	75	97
gb/st-c	45	47	52	44

TABLE 1.—*Continued*

F_1 female from cross $a \times b$ or $ab \times +$	F_2 males			
	+	*a*	*b*	*ab* (with phenotype)
gb/st-d	75	68	78	54
gb/tl	60	61	73	69
gb/+	472	408		
or/pl	63	67	154	—
or/rd	105	107	88	60 oyster
or/$R^{st\text{-}426}$	42	38	45	54 oyster
or/st-d	109	90	84	70 light peach
or/tl	53	120	—	29 white
or/to^{mz}	59	119	59	—
or/+	117	99		
pl/rd	78	120	85	—
pl/$R^{st\text{-}DR}$	92	137	89	—
pl/st-c	86	187	85	—
pl/tl	56	155	70	—
pl/to^{mz}	65	173	64	—
pl/+	178	166		
rd/$R^{st\text{-}DR}$	86	83	86	81 orange
rd/st-c	53	54	56	54 orange
rd/st-d	94	124	109	122 orange
rd/tl	217	196	353	—
rd/+	154	157		
$R^{st\text{-}DR}$*/st-c*	76	224	—	—
$R^{st\text{-}DR}$*/st-d*	93	283	—	—
$R^{st\text{-}DR}$*/tl*	80	81	86	96 light peach
$R^{st\text{-}DR}$*/*to^{mz}	87	96	72	70 orange
$R^{st\text{-}DR}$*/+*	135	124		
st-c/st-d	109	307		
st-c/tl	69	76	56	79 oyster
st-c/to^{mz}	76	73	72	77 orange
st-c/+	167	149		
st-d/tl	56	70	73	61 light peach
st-d/to^{mz}	109	79	71	73 orange
st-d/+	106	114		
tl/to^{mz}	67	70	67	43 oyster
tl/+	85	98		
to^{mz}*/+*	138	133		

st-d: scarlet-d. KAYHART 1953. Induced by slow neutrons. Phenotypically identical to scarlet-DR.2:L-11.

tl: tile. KAYHART 1953. Induced by X-rays. Eye rust-red. 3:D-12.

$tl^{oy\text{-}b}$: tile-oyster-b. WHITING 1954. Spontaneous from wild type. Eye color oyster-white; phenotypically identical to *pl* and epistatic to all other eye colors. Complementary to *tl*. 4:A-7.

to: tomato. 4:F-12. (WHITING 1951).

to^{ta} tomato-tanagra. SAUL 1952. Induced by X-rays. Dark red eye color approaches wild type with increasing age. Female sterile. Viability low. 7:J-9.

to^{mz}: tomato-manzanita. WHITING 1953. Spontaneous from wild type. Eye color dark red, slightly browner than *to*^{ta} in color. 7:L-10.

to^{mo}: tomato-moroccan. KAYHART 1953. Induced by slow neutrons. Eye color dark red, but lighter than *to*^{ta}. 5:K-11.

to$^{mo\text{-}2}$: tomato-moroccan-2. KAYHART 1953. Induced by slow neutrons. Eye color overlaps *to*^{mo} in adults but is slightly lighter in pupae.

Wild type: 8:H-8.

RESULTS OF LINKAGE TESTS

As shown in the preceding list, 15 loci are now recognized in Mormoniella. Mutants at 14 of these have been tested for linkage; *gr* has not been tested due to difficulties in establishing it in stock. For similar reasons *bl* has not been crossed with genes at all known loci. Table 1 gives results of tests which show no linkage; Table 2 gives results of tests which indicate that the genes crossed are linked. In the left-hand columns of the tables are genotypes of F_1 females from the crosses of each gene with each succeeding gene: *a*/*b* means that the female is heterozygous for *a* and *b* and that the cross was in repulsion, whereas *ab*/+ means that the cross was made in coupling. The four columns of F_2 males are arranged to show the wild type (+), mutant type *a*, mutant type *b*, and the double mutant *ab*. Phenotypes of double eye color mutants are given when distinct from either single mutant type.

In most cases, only one mutant at a locus was used to test the locus for linkage. This mutant was selected on the basis of high viability and a phenotype easily distinguished from wild type and (if possible) from other mutants used in the crosses. A few additional crosses, not included in the tables, indicated that linkage values were unchanged when other alleles at linked loci were crossed; the one exception to this (alleles at the *Ga* locus) is reported and discussed below.

TABLE 2

Linkage of traits in Mormoniella. See legend of table 1 for explanation of organization of table

F_1 female from cross *a* × *b* or *ab* × +	F_2 males			
	+	*a*	*b*	*ab* (with phenotype)
bl/*gb*	51	—	244	—
ga/*tl*	106	321	357	88 oyster
ga tl/+	124	20	22	95 oyster
ga$^{st\text{-}b}$/*tl*	21	155	172	17 light peach
ga$^{st\text{-}b}$*tl*/+	160	17	22	182 light peach
gl/*R*$^{st\text{-}DR}$	4	247	230	8
gb/*rd*	28	165	158	41
gb/*to*^{mz}	84	240	200	84
gb to^{mz}/+	190	50	42	149
or/*st-c*	21	290	278	14 light peach
or st-c/+	200	7	4	159 light peach
pl/*st-d*	65	317	261	—
pl st-d/+	97	110	20	—
rd/*to*^{mz}	15	—	547	5 light peach
rd to^{mz}/+	220	—	9	122 light peach

The data in the tables indicate that there are five groups of linked factors and a single locus (*Bk*) not included in any of the linkage groups. When linkage is calculated as described above, the groups are as follows:

1. *R* — 10.6 — *Pu* — 2.4 — *Gl*
2. *To* — 3.1 — *Rd* — 17.6 — *Gb* — 34.6 — *Bl*; *Rd* — 24.4 — *Bl*
3. *St-c* — 4.2 — *Or*
4. *Pl* — 18.5 — *St-d*
5. *Tl* — (19.0 or 10.3) — *Ga*
6. *Bk*

Distances are in Morgan Units. *Rd* and *Bl*, and *To* and *Bl* recombined at random. The *R-Pu* linkage was calculated from the data of WHITING (1950).

If *ga* is crossed with *tl*, 19.0% recombination is observed; if $ga^{st\text{-}b}$ is used in the cross, 10.3% recombination results. These percentages of recombination are almost unchanged if other alleles at the *Tl* locus are used in the crosses. It is therefore possible that the $ga^{st\text{-}b}$ stock contains a crossover suppressor linked with the *Ga* locus.

The cross of $tl^{oy\text{-}b}$ with *tl* gives wild type F_1 females; these females, unmated, produce only $tl^{oy\text{-}b}$ and *tl* F_2 males. It is possible that $tl^{oy\text{-}b}$ and *tl* are at separate, completely linked loci. It is also possible that they are at one complex locus and show the complementary allelism that has been described for the *R* locus. This second possibility is the basis of the nomenclature adopted here.

The factor for purple body color shows about 10.6% recombination with the *R* locus if the wild type allele for glass eye is present. When *gl* is present, the *R-Pu* recombinations drop to about 2.1% (19 in a culture of 897). The *R* locus and glass show about 2.4% recombination; recombinations between *gl* and *pu* occur in less than 1% of the possible cases. It is suggested by DR. P. W. WHITING (1955) that "*gl*" may be an inversion, and that the *Pu* locus may be included within the inversion. Cytological observations on *gl*/+ females have not yet been made. The viability of *gl* males is about equal to that of + males, and *gl*/+ females have high fecundity. The fecundity of homozygous *gl* females is very low, however.

Spermatogonial cells of Mormoniella contain five chromosomes, the haploid number for the species. Although this study has shown the existence of five linkage groups and one factor, black, segregating independently of all other loci, it is impossible to determine at present whether the five linkage groups are located on five separate chromosomes.

SUMMARY

1. New mutants in Mormoniella are described and discussed. A system for describing and naming eye color mutants is introduced; this system is based on the use of Maerz-Paul color charts.

2. The known loci are shown to be associated in five linkage groups as shown on page 936.

3. Data obtained by the use of *gl* stock are discussed. These data support a suggestion by WHITING that "glass" is an inversion which may include the Purple locus.

4. It is possible that the Tile locus is complex, containing factors which show complementary allelism.

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THE RELATION BETWEEN EXPRESSIVITY AND SELECTION AGAINST EYELESS IN DROSOPHILA MELANOGASTER[1]

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MANY mutant stocks of Drosophila mass cultured in the laboratory for generations become phenotypically almost indistinguishable from wild type. Upon outcrossing, the mutant reappears in the F_2 with its pristine strength of expression. Presumably, genes reducing the degree of expression, or expressivity, of the major character of the mutant stock had appeared and accumulated through natural selection during its maintenance in the laboratory. These modifying genes are probably disadvantageous to their possessors in a culture of wild type flies. MARSHALL and MULLER (1917) found more extreme phenotypes in homozygous recessive mutants obtained from stocks maintained in a heterozygous condition for many generations, than in parallel stocks maintained as homozygotes.

Does lower expressivity reduce the rate of selection against a deleterious gene when compared with its wild type allele? If so, is the relation demonstrable within a phenotypically variable isogenic stock or must genetic modifiers exist? The rate of selection is determined by the viability and productivity of flies of the relevant genotypes. The first of these parameters can be measured in crosses giving equal numbers of mutant and wild type zygotes whereas the latter parameter can be estimated from experiments in which mutant and wild type males compete in mating, or from experiments in which mutant and wild type females compete in oviposition. The effect of the degree of expression of the mutant on viability and productivity—and thus on selection—can be studied in crosses involving mutant parents with different grades of expression.

The next question is whether any correlation observed between expressivity and selection is to be attributed to the accumulation of modifiers. If the degree of expression is influenced by modifiers, there should be a positive correlation between the expression of the mutant character in parent and offspring. Furthermore, if the character is bilateral, the grades on the two sides are correlated to a greater extent within the progeny of a group of parents heterozygous for modifiers than of homozygous parents. The reason for this is that with heterozygous parents both local environment and genetic segregation affect the individual offspring whereas with homozygous parents only the local environment determines differences in expression.

The results to be reported demonstrate that modifiers soon disrupted the initial isogenicity of the mutant stock, enhancing its viability and productivity and decreasing its expressivity, so long as the mutant stock was maintained separately from the wild type stock. Once mutant and wild type parents were taken from segregating cultures, the accumulation of modifiers was greatly retarded. Neverthe-

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less, it is clearly demonstrable that mutant males and females of extreme expressivity are less productive than mutants of more nearly wild type appearance. No effect of expressivity on viability was found.

MATERIALS AND METHODS

The eyeless-4 (ey^4) allele was used because of its variable expression. For brevity, it will be referred to simply as *ey* hereafter. The selective disadvantage of *ey/ey* flies with various degrees of reduction in eye size were compared against a "wild type" standard. Two stocks were derived, differing only in their fourth chromosomes. For the + stock, a *Cy/Pm*; *Mo Sb/H* ♂ was crossed first to a *ClB/dl-49* ♀ and then to one of his Bar Curly Stubble daughters. From the progeny of the latter cross a Curly Stubble male and a Bar Curly Stubble female were selected for mating. Wild type progeny should be isogenic for chromosome X (from the *Cy/Pm*; *Mo Sb/H*♂) and chromosomes 2 and 3 (from the *ClB/dl-49* ♀). Of the several fertile strains of wild type appearance so obtained, one was used in this study.

The *ey* chromosome was introduced from flies received in December 1952 from the California Institute of Technology. An *ey/ey* ♂ was mated to a *ClB/dl-49*; *Cy/Pm*; *Mo Sb/H* ♀. An F_1 *dl-49*; *Pm/+*; *H/+*; *+/ey* ♂ was mated to an F_1 *ClB/+*; *Cy/+*; *Mo Sb/+*; *+/ey* ♀. An F_3 *dl-49*; *Cy/Pm*; *Mo Sb/H*; *ey/ey* ♂ was then selected for mating with a female from the isogenic wild type stock, from which eventually an *ey* stock was extracted whose chromosomes X, 2 and 3 were as isogenic with the + stock as can be insured by the use of stocks bearing these marked inversions. To secure reasonable isogenicity in chromosome 4, a +/+ ♂ was mated successively to an *ey/ey* ♀ and to one of his *+/ey* daughters. The progeny of the latter cross were pair-mated to extract the *ey* strain used in the study. Five generations of brother-sister pair matings of the + strain raise to 60% the chance that the wild type fourth chromosomes were isogenic. The use of marked inversions does not guarantee isogenicity because of the enhancement of crossing over elsewhere in flies heterozygous for rearrangements. The lack of parent-offspring or right-left correlations soon after derivation of the stocks (see below), however, strongly suggests that isogenicity was achieved.

The *ey* stock so obtained had much smaller eyes than the original *ey* stock, varying with temperature but not overlapping wild type. The *ey/ey* flies used as parents were classed by eye size as follows: "small"—with both eyes less than one third normal; "medium"—both approximately half normal; "large"—both over half normal, at least one more than two thirds normal; and "asymmetric"—the two eyes of markedly different size. Intermediates between these categories were not used.

Each eye of the offspring from all crosses was graded according to the arbitrary system:

Grade	Amount of eye
0	no more than one facet
1	less than one third of an eye
2	between one third and two thirds of an eye
3	between two thirds of an eye and a full eye
4	normal wild type eye

In statistical treatment of phenotypic grade, except in correlating right and left eyes, the individual offspring was represented by the sum of the grades of his two eyes. Thus, zero represented a totally eyeless individual and 8, a wild type individual, while 4 represented individuals with both eyes grade 2, with one eye grade 3 and the other grade 1, or with one eye normal and the other missing (these usually breeding as $+/ey$ and thus haplo-IV on one side of the head).

The experimental crosses were performed in half-pint bottles containing the standard cornmeal-agar-Karo-molasses medium, each containing eight pairs of parents. Under these conditions, the eclosion rate drops sharply on the fourth or fifth day. Genetically *ey/ey* flies eclosing later have larger eyes. Eclosion began at approximately the same time in all bottles mated at the same time. In two series, counts were made daily during the entire eclosion period. Both the rate at which the average grade increased and the day on which the highest grade of *ey/ey* occurred differed between the two series. However, within each series the different parental classes and kinds of matings had no influence on either rate of increase or date of maximum eclosion. In cultures producing both $+/ey$ and *ey/ey* offspring, the relative frequencies of these classes did not alter systematically or significantly throughout the eclosion period.

In most of the study, offspring were counted which emerged during maximum eclosion, from approximately 60 to 78 hours after the first eclosion. In the earlier part (series 8–40 as described in the next section), all offspring emerging during this period were classified. Later (series 41–50), no more than 30 flies of each sex were classified, since χ^2 tests on the series 39 counts showed the sampling to be random. When less than 20 emerged, the remainder of the 20 were classified the next day. Cultures producing fewer than 20 offspring during two days of counting were omitted.

EXPERIMENTAL DESIGN

Selection acts through both viability and productivity, or in other words, for an insect, through both survival from zygote to imago, and contribution of either egg or sperm to the zygotes of the next generation. The relative viabilities of mutant and wild type genotypes can be expressed by a viability coefficient s: if the zygotic ratio is 1 mutant: 1 wild type, the final adult ratio is $(1 + s)$ mutants: 1 wild type. The productivity coefficient used here is defined so that $(1 + t)$ eggs are laid (or fertilized) by the mutant for every egg laid (or fertilized) by the wild type of the same sex.

The relative viability of $+/ey$ and each class of *ey/ey* flies was evaluated from the progeny of two systems of mating: 8 *ey/ey* ♀♀ × 8 $+/ey$ ♂♂ per bottle (mating system A) and 8 $+/ey$ ♀♀ × 8 *ey/ey* ♂♂ per bottle (mating system B). From the reciprocal crosses, any maternal influence on viability, or non-virginity of parental females could be assessed. Germinal selection was assumed negligible, i.e., the zygotic ratio was assumed to be $\frac{1}{2}ey/ey$: $\frac{1}{2}+/ey$, since MULLER and SETTLES (1927) demonstrated no selection against even a deficiency for several autosomal loci. Reports of germinal selection (LOBASHOV 1940) might simply result from larval selection.

The relative productivities of *ey/ey* and $+/ey$ ♂♂ were determined from the progeny of mating system C, 8 *ey/ey* ♀♀ × (4 *ey/ey* ♂♂ + 4 $+/ey$ ♂♂) per bottle. The relative productivities of *ey/ey* and $+/ey$ ♀♀ were determined from the

progeny of mating system D, (4 *ey/ey* ♀♀ + 4 +/*ey* ♀♀) × 8 *ey/ey* ♂♂ per bottle. Since progeny from any bottle were sampled just once at maximum eclosion, only one of the two components of productivity was measurable: rate, not duration, of offspring production. A comprehensive estimate of productivity would include both.

A final mating system, E, was 8 *ey/ey* ♀♀ × 8 *ey/ey* ♂♂ per bottle.

Bottles were grouped in series started at different times. The parents in any one series were of the same age from the same sources, handled identically. Most series included:

Phenotype of *ey* parents	Mating system				
	A	B	C	D	E
small	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
medium	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
large	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
asymmetric	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles

In later series, there were three instead of two replicates. Parents were removed and examined again before their offspring eclosed.

For the first 23 series, the +/*ey* parents came from isogenic + ♂♂ × *ey/ey* ♀♀. The *ey/ey* parents were selected from the isogenic stock. All flies used as parents eclosed within 12 to 18 hours after removal of all imagoes from stock bottles. Before mating, males and females were aged in vials containing 20–100 of the same sex. Vials producing eggs or larvae were discarded.

The isogenic + stock was accidentally lost after series 23. For the next 16 series, +/*ey* flies came from mating systems A and B of preceding series. From series 27 on, *ey/ey* parents were obtained from mating systems A through E (E avoided as much as possible to retard any accumulation of modifiers). Meanwhile, a + stock was re-isolated from +/*ey* offspring of series 20. Males from this later + stock, crossed to *ey/ey* ♀♀ of assorted phenotypes, produced +/*ey* parents for the last 11 series.

Series 1 through 7 were run at 29°C for maximal phenotypic variation. The sterility in most of these was excessive, rendering a detailed statistical comparison with later series fruitless. Series 2, however, yielded enough offspring to calculate certain correlations. Fertility was improved by running series 8–50 at 25°C. Series were begun only a few days apart, except for series 21, 57 days after series 20.

FACTORS INFLUENCING EYE SIZE IN EY/EY STOCKS

The stocks did not long remain isogenic. Instead, eye size varied more markedly in the later series.

The eye grades of *ey/ey* offspring in each bottle were averaged. Table 1 presents the mean of these averages for each group of bottles with the same phenotypic class of *ey* parent and mating system (A or E), in series 8–20 (October to December, 1953), in series 21–33 (February to early April, 1954), in series 34–42 (late April and May, 1954), and in series 43–50 (June and July, 1954). The distributions of eye grades are also given.

TABLE 1

Percentage of ey offspring with each summed eye grade, and average of bottle mean eye grades, in mating systems A and E, grouped by series and parent phenotype

Mating system	Series	Phenotype of *ey* parent	Male offspring							Female offspring						
			Number offsp.	Summed eye grade					Mean	Number offsp.	Summed eye grade					Mean
				<2	2	3	4	>4			<2	2	3	4	>4	
A	8–20	small	534	10	34	29	20	7	2.78	444	21	38	25	13	3	2.29
ey/ey ♀♀ ×		medium	478	6	36	33	21	4	2.79	466	16	36	30	14	4	2.43
+/ey ♂♂		large	325	5	35	33	23	4	2.85	318	16	35	27	15	7	2.65
		asymmetric	462	10	35	30	21	4	2.72	388	23	40	23	12	2	2.31
	21–33	small	450	5	29	31	28	7	3.02	428	18	27	28	20	7	2.72
		medium	439	3	16	30	36	15	3.48	345	9	18	25	33	15	3.35
		large	371	1	15	27	38	19	3.65	339	10	15	23	30	22	3.50
		asymmetric	413	3	21	29	35	12	3.30	356	9	23	29	27	12	3.16
	34–42	small	431	3	31	33	36	6	3.04	428	15	27	30	21	7	2.83
		medium	433	3	15	29	37	16	3.55	378	7	18	29	30	16	3.33
		large	422	1	11	28	38	22	3.78	383	6	12	25	31	26	3.69
		asymmetric	391	2	18	27	35	18	3.51	364	9	21	24	28	18	3.28
	43–50	small	320	4	34	33	24	5	2.95	314	11	31	28	23	7	2.85
		medium	327	3	28	25	31	13	3.29	333	5	21	27	29	18	3.44
		large	343	2	19	23	35	21	3.66	322	5	14	22	33	26	3.75
		asymmetric	302	2	25	33	28	12	3.27	341	9	22	27	29	13	3.19
E	8–20	small	1081	13	50	25	12	0	2.38	1037	34	39	21	6	0	1.94
ey/ey ♀♀ ×		medium	951	6	41	33	19	1	2.71	945	20	39	28	12	1	2.33
ey/ey ♂♂		large	656	4	37	32	25	2	2.87	641	14	33	32	17	4	2.58
		asymmetric	962	8	41	31	19	1	2.65	871	22	40	26	11	1	2.22
	21–33	small	834	5	36	39	17	3	2.96	814	18	33	28	18	3	2.54
		medium	959	3	13	28	41	15	3.62	905	9	20	25	33	13	3.26
		large	596	1	5	18	44	32	4.20	547	3	10	17	37	33	4.04
		asymmetric	721	2	18	32	36	12	3.43	683	10	23	28	28	11	3.08
	34–42	small	790	6	31	33	26	4	2.93	811	17	30	29	20	4	2.64
		medium	772	2	16	25	37	20	3.63	694	5	15	26	34	20	3.55
		large	931	1	8	21	42	28	4.02	851	5	14	19	36	26	3.83
		asymmetric	854	2	20	28	36	14	3.43	804	6	23	27	30	14	3.30
	43–50	small	644	6	38	32	22	2	2.77	657	18	31	30	18	3	2.55
		medium	688	2	17	25	41	15	3.54	676	4	16	27	38	15	3.50
		large	613	0	9	16	44	31	4.14	624	3	8	18	31	40	4.20
		asymmetric	636	4	21	30	33	12	3.32	652	7	21	30	32	10	3.17

After the first group of series, the mean eye grade increased, especially when parents were "medium" or "large". The offspring were more extreme—smaller-eyed from "small" parents and larger-eyed from "large" parents—when both parents were *ey* and alike (mating system E) than when one parent was *+/ey* (mating system A). The "within bottle" variance increased concurrently, possibly because of the appearance and segregation of modifiers after series 20, but possibly simply as an artifact of the grading scale.

The initial isogenicity of the *ey* stock is attested by the offspring of mating system E in series 2. The midparent-son correlation (based on the offspring of "small", "medium" and "large" parents) was $-.101$, which is not significantly different from zero (d.f. = 163, $P = .20$). The midparent-daughter correlation was $-.018$, also not significantly different from zero (d.f. = 159, $P = .81$).

Before or during series 8–20, modifiers of the *ey* eye size had appeared. The parent-offspring correlations (table 2) are considerably lower in series 8–20 than they are

TABLE 2

Coefficients of correlation between grade of ey *parent* and grade of individual* ey *offspring, mating systems A through E. Equal replication within series*

Mating system	Series	Sons		Daughters	
		Number offsp.	r	Number offsp.	r
A	8–20	686	.1113	639	.1153
ey/ey ♀♀ × +/*ey* ♂♂	21–33	1054	.2299	926	.2570
	34–42	1295	.2702	1207	.2775
	43–50	958	.2597	932	.2921
B	8–20	892	.1960	855	.1320
+/*ey* ♀♀ × *ey/ey* ♂♂	21–33	1176	.1605	1089	.1614
	34–42	1077	.2089	1021	.2426
	43–50	982	.2203	910	.1955
C	8–20	1047	.1335	911	.2195
ey/ey ♀♀ × +/*ey* ♂♂ & *ey/ey* ♂♂	21–33	1436	.2644	1385	.2893
	34–42	1702	.3307	1618	.3725
	43–50	1412	.3448	1374	.3866
				1174	.1580
D	8–20	1324	.0960		
+/*ey* & *ey/ey* ♀♀ × *ey/ey* ♂♂	21–33	1824	.3276	1624	.3003
	34–42	1766	.3280	1668	.3391
	43–50	1445	.3917	1485	.4203
E	8–20	1463	.1793	1467	.2223
ey/ey ♀♀ × *ey/ey* ♂♂	21–33	1914	.4688	1729	.4415
	34–42	2481	.3770	2363	.3660
	43–50	1945	.4584	1957	.4742

* Average grade of:

	ey mothers			*ey* fathers		
	Small	Medium	Large	Small	Medium	Large
series 8–20.....	1.38	4.0	5.5	1.76	4.0	5.5
series 21–33.....	1.41	4.0	5.5	1.78	4.0	5.5
series 34–42.....	1.53	4.0	5.5	1.81	4.0	5.5
series 43–50.....	1.54	4.0	5.5	1.85	4.0	5.5

later, but are already significantly different from zero. In the interval before series 21–33, heterogeneity due to modifiers was heightened. The genetic variance increased 5.3-fold (= $[.46/.20]^2$) from the first to the fourth group of series; the original isogenic stock had none.

As would be expected if phenotypic grade is influenced by modifiers, the correlations are highest in mating system E and lowest in A and B. The differences in correlations found among the mating systems are less in the earlier than in the later series. Mating system A does not differ significantly from B within a series group, nor C from D. Apparent discrepancies—parent-son correlations in series 8–20 for B and D, and

TABLE 3

Correlation coefficients of right and left eye grades in ey *offspring for each phenotypic class of parent in each mating system.* ($618 < N < 2104$)

Mating system	Series	Phenotypic class of *ey* parent			
		Small	Medium	Large	Asymmetric
A	8–20	.316	.230	.293	.163
ey/ey ♀♀ × *+/ey* ♂♂	21–33	.283	.330	.397	.335
	34–42	.325	.432	.408	.437
	43–50	.361	.491	.476	.391
B	8–20	.248	.211	.219	.221
+/ey ♀♀ × *ey/ey* ♂♂	21–33	.292	.362	.503	.320
	34–42	.385	.417	.436	.397
	43–50	.380	.360	.447	.439
C	8–20	.295	.280	.228	.334
ey/ey ♀♀ × *+/ey* & *ey/ey* ♂♂	21–33	.270	.341	.406	.364
	34–42	.302	.408	.422	.443
	43–50	.302	.440	.474	.557
D	8–20	.199	.222	.228	.227
+/ey & *ey/ey* ♀♀ × *ey/ey* ♂♂	21–33	.277	.326	.434	.334
	34–42	.289	.409	.434	.356
	43–50	.357	.409	.478	.405
E	8–20	.222	.215	.279	.243
ey/ey ♀♀ × *ey/ey* ♂♂	21–33	.223	.290	.363	.241
	34–42	.314	.366	.411	.380
	43–50	.276	.384	.469	.351

parent-daughter correlations in series 43–50 for A and B—are of borderline significance only, probably attributable to accidents of sampling.

"Asymmetric" parents, with an average grade somewhat less than 4.0, seemed genetically similar to "medium" parents. The variance of the offspring eye grades between bottles with "asymmetric" parents was not significantly different from that with "medium" parents in the same groups of series. Furthermore, the correlation between right and left eyes in offspring of "asymmetric" parents is about the same as in offspring of "medium" parents (table 3).

A significant right-left correlation does not necessarily indicate segregation of modifiers, since non-genetic factors could conceivably affect both eyes simultaneously. An increase in right-left correlation with time does, however, indicate an increased number of modifiers segregating.

The right-left correlation was negligible—+.08 for males ($.4 < P < .5$) and −.22 for females ($.02 < P < .05$)—in the isogenic *ey* stock when first derived. A significant correlation was found, however, in the offspring from each mating system, for each parent phenotype, and in each series group in the experiment (table 3). Differences in r of 0.12 are significant at least at the 5% level; differences of 0.15, at the 1% level.

The increase in right-left eye correlation with time is pronounced, though somewhat less so in the offspring of "small" parents in mating system E, suggesting that "medium" and "large" parents are more heterozygous for modifiers affecting eye size than "small" parents.

From the pattern of parent-offspring and right-left correlations, the history of the eye-size modifiers of *ey* can be reconstructed. Initially, the *ey* and + stocks differed at most in their fourth chromosomes. The + fourth chromosomes probably came from a single source, but possibly two. Between the time of the marked-chromosome derivation of the first *ey* stock in August 1953 and the first group of series at 25°C (series 8–20, October through December), plus modifiers arose. Genetic diversity increased almost 5-fold following the 57-day interval between series 20 and 21, with no consistent increase during the last two periods (April through July).

Over 9000 flies were used to propagate the stocks after their derivation by the method which GOWEN, STADLER and JOHNSON (1946) found reduced heterozygosity more effectively than 97 generations of brother-sister pairing. Any locus with a mutation rate of 10^{-5}/chromosome-generation had a 9% chance of mutating during the experiment. Several modifying mutations were likely, although probably only a modest fraction of the estimated 5000 loci of *melanogaster* influence the *ey* phenotype. All four chromosomes can carry eyeless modifiers (BARON 1935). Any mutation lengthening the larval period would correspondingly increase facet number (BODENSTEIN 1939).

Until series 27, *ey* parents of all classes came from the same stock bottles. Meanwhile, larger-eyed flies became more common; natural selection was favoring plus-modifiers. From series 27 on, *ey* parents were taken from experimental cultures of mating systems A, B, C and D and randomized as to source. Thus, while few were the result of assortative matings, the earlier selection within *ey* stocks for increased eye-size was replaced by selection within each phenotypic grade of eyeless, from competition with $+/ey$.

RELATIVE VIABILITIES OF EY/EY AND +/EY

Table 4 gives the average percentage of $+/ey$ offspring per bottle of mating systems A and B for each class of *ey* parent, the χ^2's of heterogeneity between bottles, their degrees of freedom and the probabilities of obtaining higher χ^2's without real heterogeneity. Table 5 gives the tests of differences between the categories itemized in table 4. A single bottle, mating system B, "asymmetric", series 24, gave a highly aberrant ratio in its sons (5 ey/ey : 30 $+/ey$), although the ratio in its daughters was normal, with *ey* daughters slightly in excess. This bottle was excluded from the statistical treatments.

Evidently, it is not eye size of the *ey* parents which affects viability. However, in series 8–30, significantly smaller proportions of daughters than of sons are ey/ey. There is little heterogeneity in the daughters. The mating-system difference in daughters of "small" parents is 2.55 times its standard error, and might have the same cause as the mating-system difference in sons (see below). The daughters of "asymmetric" parents differ significantly from early to late series—$\chi^2 = 7.2$, $.001 < P < .01$, in mating system A and $\chi^2 = 4.2$, $.02 < P < .05$, in mating system B.

TABLE 4

Percentage of heterozygotes in mating systems A and B

Offspring sex	Phenotype of *ey* parent	Series	Mating system A (*ey/ey* ♀♀ × +/*ey* ♂♂)					Mating system B (+/*ey* ♀♀ × *ey/ey* ♂♂)				
			Number offsp.	Average % +/*ey* in offspring	χ^2	d.f.	*P*	Number offsp.	Average % +/*ey* in offspring	χ^2	d.f.	*P*
♂♂	small	8–30	1785	51.88	46.10	45	.3–.5	1668	55.76	48.50	41	.1–.2
		31–50	1922	52.71	51.30	53	.5–.7	1623	53.60	54.34	50	.3–.5
	medium	8–30	1554	52.06	48.55	43	.2–.3	1781	54.74	26.72	44	.95–.98
		31–50	1948	52.98	48.89	54	.5–.7	1669	53.09	66.07	54	.1–.2
	large	8–30	1189	53.15	24.72	29	.5–.7	1602	56.37	39.00	35	.2–.3
		31–50	1902	52.73	57.01	54	.3–.5	1751	54.65	54.02	53	.3–.5
	asymmetric	8–30	1349	51.96	38.78	35	.3–.5	1252	55.43	29.74	30	.3–.5
		31–50	1818	54.07	45.64	53	.7–.8	1854	53.34	66.10	53	.1–.2
	Total	8–30	5877	52.20	158.15	152	.3–.5	6303	55.56	143.96	150	.5–.7
		31–50	7590	53.11	202.84	214	.5–.7	6897	53.68	240.53	210	.05–.1
♀♀	small	8–30	1649	53.37	45.26	45	.3–.5	1715	56.09	49.77	42	.2–.3
		31–50	1830	54.48	59.21	53	.2–.3	1638	58.00	48.21	52	.5–.7
	medium	8–30	1565	55.02	46.05	43	.3–.5	1732	54.79	47.76	45	.3–.5
		31–50	1763	55.64	62.26	53	.1–.2	1645	55.32	68.67	53	.05–.1
	large	8–30	1230	55.37	26.55	29	.5–.7	1452	55.30	47.95	35	.05–.1
		31–50	1810	54.75	64.02	54	.1–.2	1632	54.90	44.22	53	.7–.8
	asymmetric	8–30	1359	57.32	41.56	38	.3–.5	1200	52.58	19.61	31	.9–.95
		31–50	1696	52.48	45.99	53	.7–.8	1735	56.43	50.21	53	.5–.7
	Total	8–30	5803	55.16	159.42	155	.3–.5	6099	54.85	165.09	153	.2–.3
		31–50	7099	54.36	231.48	213	.1–.2	6650	56.17	211.32	211	.3–.5

Between mating systems A and B within the earlier series with "asymmetric" parents, $\chi^2 = 5.78$; within the later series, $\chi^2 = 5.42$, $.01 < P < .02$. However, which mating system has the higher proportion of +/*ey* daughters of "asymmetrics" differs from early to later series, so that, pooling mating systems, the net early-late difference is negligible ($\chi^2 = .23$), and, pooling groups of series, the net mating-system difference is also negligible ($\chi^2 = .03$).

The viability of *ey/ey* sons is very significantly greater in mating system B than in mating system A in series 8–30. The only tenable explanation invokes the genetic heterogeneity demonstrated even in the earlier series. Modifiers favoring viability of the *ey/ey* may well have accumulated during the separate maintenance of *ey* and + stocks before series 24. Some modifiers could act via a maternal effect on egg cytoplasm. Maternal genotype has been shown to influence viability of certain offspring genotypes (LYNCH 1920; REDFIELD 1926). Thus, it is conceivable that *ey/ey* zygotes survive better if their mothers came from the *ey* stock (mating system A) than if their mothers came from a cross to + (mating system B). For the effect to be more pronounced in sons than in daughters, chromosome X must have the same effect on viability in both stocks, but must override the earlier conditioning of the egg cytoplasm only when paired, as in females. By series 27, *ey/ey* mothers (mating system A) and +/*ey* mothers (mating system B) would differ systematically only in chromosome 4. The preponderance of inheritance from the *ey* stock would account for the later series resembling the earlier series of mating system A more than mating system B.

An unlikely alternative explanation is that not all the mothers used were virgin.

TABLE 5

Chi square analysis of proportions of heterozygotes in mating systems A and B

Comparison	Groupings for which estimates of % of + /*ey* are accepted	χ^2	d.f.	*P*
Phenotype, series group, sex and mating system	Total of all 32 classes	50.8	31	.01–.02
Phenotype	A ♂♂ 8–30, A ♂♂ 31–50, B ♂♂ 8–30, B ♂♂ 31–50, A ♀♀ 8–30, A ♀♀ 31–50, B ♀♀ 8–30, B ♀♀ 31–50	19.3[a]	24	.70–.80
Series group, sex and mating system	Total of 32 classes, applied to the 8 groups pooling phenotypes	31.5	7	≪.001
Early vs. late series groups	A ♂♂, B ♂♂, A ♀♀, B ♀♀, 8 groups	8.9[b]	4	.05–.10
♂♂ vs. ♀♀	A 8–30, A 31–50, B 8–30, B 31–50, 8 groups	21.7[c]	4	≪.001
A vs. B	♂♂ 8–30, ♂♂ 31–50, ♀♀ 8–30, ♀♀ 31–50, 8 groups	18.9[d]	4	<.001
(a) Components of phenotype	A ♂♂ series 8–30	0.6	3	.90–.95
	A ♂♂ series 31–50	0.9	3	.80–.90
	B ♂♂ series 8–30	0.9	3	.80–.90
	B ♂♂ series 31–50	1.0	3	.80–.90
	A ♀♀ series 8–30	4.7	3	.10–.20
	A ♀♀ series 31–50	3.7	3	.20–.30
	B ♀♀ series 8–30	3.7	3	.20–.30
	B ♀♀ series 31–50	3.8	3	.20–.30
(b) Components of early vs. late (pooled phenotypes)	A ♂♂	1.2	1	.20–.30
	B ♂♂	4.7	1	.02–.05
	A ♀♀	0.8	1	.30–.50
	B ♀♀	2.2	1	.10–.20
(c) Components of ♂♂ vs. ♀♀ (pooled phenotypes)	A series 8–30	10.3	1	.001–.01
	A series 31–50	2.3	1	.10–.20
	B series 8–30	0.6	1	.30–.50
	B series 31–50	8.5	1	.001–.01
(d) Components of A vs. B (pooled phenotypes)	♂♂ series 8–30	13.8	1	≪.001
	♂♂ series 31–50	0.4	1	.50–.70
	♀♀ series 8–30	0.1	1	.70–.80
	♀♀ series 31–50	4.5	1	.02–.05

If this were so, the non-virginity existed chiefly in the +/*ey* mothers in the earlier series. To account quantitatively for the discrepancy, 10.8% of the sperm fertilizing the eggs of these +/*ey* ♀♀ must have come from their brothers. Such a high value for a single class of females is extremely unlikely, since the procedure for obtaining virgins was uniform throughout the experiment. The only direct evidence of non-virginity of mothers of any class could be obtained from +/*ey* offspring in bottles belonging to mating system E after series 26, when *ey* parents came from bottles also

TABLE 6

Viability coefficients s *for* ey/ey

Sex	Mating system	Series	Number +/*ey* (*n*)	Total number (*N*)	s*	95% Confidence limits	
						Upper	Lower
♂♂	A	8–30	3068	5877	−.077	−.028	−.123
		31–50	4031	7590	−.109	−.068	−.149
	B	8–30	3502	6803	−.193	−.152	−.232
		31–50	3702	6897	−.129	−.087	−.170
	Pooled		14303	26667	−.128		
♀♀	A	8–30	3201	5803	−.180	−.136	−.221
		31–50	3859	7099	−.153	−.112	−.192
	B	8–30	3345	6099	−.169	−.126	−.210
		31–50	3735	6650	−.212	−.173	−.250
	Pooled		14140	25651	−.179		

* $s = \dfrac{N}{n(1-a)} - 2$, where a is the proportion of wild type appearing individuals which are genetically *ey/ey*.

producing +/*ey*. Almost any non-virginity would be due to precocity of brothers. In these series (27–50), of 61 bottles with 478 "small" mothers, 5 gave one +/*ey* offspring each, totaling 3 sons and 2 daughters. They occurred in series 31, 35–37 and 50. Of 62 bottles with 496 "large" mothers, one in series 49 produced 6 sons and 3 daughters which proved +/*ey* in test-crosses. In this bottle, more than one mother may have mated previously. Of 62 bottles with 494 "medium" mothers and of 60 bottles with 472 "asymmetric" mothers, none gave + offspring. Thus probably 7 of the 1940 mothers used for mating system E in series 27–50 had mated previously with ey^+-bearing males. In consequence, 14 of their 15,419 offspring, or 0.09%, were +/*ey* because of non-virginity, i.e., roughly 0.36% of the sperm came from prior insemination, in contrast to the 10.8% required to attribute the early difference between mating systems A and B to non-virginity.

Exceptionally large-eyes *ey/ey* flies could be erroneously classed as +. To detect this, 5901 phenotypically + offspring in series 22–26, 38, and 44–50 were testcrossed individually to *ey/ey* in vials at room temperature. In 24 vials only eyeless offspring were obtained, the remainder giving both *ey* and +. The proportion of phenotypically + flies which proved to be *ey/ey* was 0.407% ± 0.082%, unaffected by sex, series, or parent phenotype. Hence, approximately 0.46% of the *ey/ey* ♂♂ appeared +, as did 0.50% of the *ey/ey* ♀♀.

If x represents the proportion of offspring which are +/*ey*, after correcting for phenotypic overlap, the viability coefficient of *ey/ey* is

$$s = \frac{1}{x} - 2.$$

Because of the maternal effect on viability, table 6 gives coefficients for each mating system in both groups of series. The overall average was −.128 for males and −.179 for females.

RELATIVE PRODUCTIVITIES OF EY/EY AND +/EY MALES

Offspring from four types of matings in mating system C yielded evidence concerning the relative productivities of *ey/ey* and +/*ey* ♂♂:

(1) 8 "small" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "small" *ey/ey* ♂♂;
(2) 8 "medium" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "medium" *ey/ey* ♂♂;
(3) 8 "large" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "large" *ey/ey* ♂♂;
(4) 8 "asymmetric" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "asymmetric" *ey/ey* ♂♂.

The productivities of the four classes of *ey/ey* fathers can be compared, using that of +/*ey* ♂♂ as standard. The ratio of +/*ey* to *ey/ey* in the progeny depends on two factors: the relative proportions of eggs fertilized by the two kinds of males and the relative viabilities of the *ey/ey* and +/*ey* offspring. The male productivity coefficient, $l^{♂}$, evaluates the first of these factors. Since the latter factor differs in sons and daughters, two estimates of $l^{♂}$ can be made, one based on sons and the other on daughters.

The unit of analysis was the bottle, rather than the individual offspring, since the χ^2 of heterogeneity of proportion of +/*ey* in sons between bottles with "medium" parents was 260.733, d.f. = 97, $P \ll .0001$. The percentages of +/*ey* in the offspring were transformed into arcsines for analyses of variance, to permit valid comparisons when percentages differed widely. Variation between bottles signifies a real variation in productivity. Table 7 presents the correlation between the proportions of +/*ey* in daughters and in sons. All correlations are significantly positive, especially between sons and daughters of "medium" parents, whose heterogeneity was tested.

The major results for mating system C are shown in table 8. The proportion of +/*ey* offspring in series 8–30 was on the average higher than in series 31–50, except for "large" parents. When a four-partite division of the series is made, the shift in proportion obviously is not gradual, but abrupt. Various explanations could be ruled out. The difference was not due to the inclusion of more series with one range of ages of parents in the earlier than in the later series, since an analysis of variance disclosed that, if male productivity depends on age at all, it is equally influenced

TABLE 7

Coefficients of correlation between ♂ and ♀ arcsin $\sqrt{\%+/ey}$ *'s in mating system C*
(ey/ey ♀♀ × +/ey ♂♂ and ey/ey ♂♂)

Phenotypic class of *ey* parents	Number of bottles (N)	Correlation between daughters & sons (r)	95% confidence limits	
			Upper	Lower
Small	96	+.500	+.636	+.332
Medium	96	+.720	+.804	+.607
Large	85	+.617	+.734	+.466
Asymmetric	94	+.692	+.784	+.569
Pooled	371	+.639	+.700	+.575

TABLE 8

Comparison of arcsin $\sqrt{\%+/ey}$ in series 8–30 and series 31–50, mating system C, for sons and for daughters

Phenotype of *ey* parents	Offspring sex	Series 8-30			Series 31-50			S.e. of diff. of means	STUDENT'S *t*	*P*
		No. bottles	Mean % +/ey	Mean arcsine	No. bottles	Mean % +/ey	Mean arcsine			
"Small"	♂♂	43	38.32	37.99	53	31.90	33.91	1.61	−2.49	.01–.02
	♀♀	43	40.05	39.51	54	35.25	36.02	1.71	−2.04	.02–.05
"Medium"	♂♂	45	33.02	34.53	53	26.45	30.10	1.77	−2.50	.01–.02
	♀♀	43	37.76	37.42	53	27.28	30.60	1.96	−3.48	.001
"Large"	♂♂	32	29.91	32.15	54	32.68	34.01	2.45	+0.76	> .05
	♀♀	31	30.88	33.00	54	32.87	34.61	2.12	+0.76	> .05
"Asymmetric"	♂♂	40	35.71	36.46	55	29.77	32.25	1.46	−2.88	.001–.01
	♀♀	39	37.99	37.68	55	30.67	33.19	1.70	−2.64	.001–.01
Pooled	♂♂	..	...	35.47	..	...	32.57	0.92	−3.17	< .001
	♀♀	..	...	37.20	..	...	33.62	1.00	−3.60	< .001

Comparison	S.e. of diff. of means	STUDENT'S *t*	*P*
♂♂ series 8–30 "small" vs. "medium"	1.71	+2.02	.02–.05
"medium" vs. "large"	2.37	+1.00	> .05
♂♂ series 31–50 "small" vs. "medium"	1.67	+2.28	.02–.05
"medium" vs. "large"	1.88	−2.08	.02–.05
♀♀ series 8–30 "small" vs. "medium"	1.88	+1.11	> .05
"medium" vs. "large"	2.26	+1.96	≃.05
♀♀ series 31–50 "small" vs. "medium"	1.49	+3.64	< .001
"medium" vs. "large"	1.50	−2.67	.001–.01

in *ey/ey* ♂♂ and in +/*ey* ♂♂ between 1 and 19 days after eclosion ($F = 0.3$, d.f. = 3 and 39, $P > .05$). The ratio of *ey/ey* to +/*ey* fathers surviving until their removal from the cultures was also not related to their measured productivities ($F < 1$, d.f. = 2 and 74, $P > .05$). The series with unusually high or low "productivity" of *ey/ey* fathers did not correspond with those displaying the highest or lowest "viabilities" of *ey/ey* offspring in mating systems A and B.

Two possible explanations remain to be discussed. Both presuppose heterogeneity at other loci influencing fertility. The first presumes that factors increasing productivity in *ey/ey* ♂♂ accumulated while the *ey* stock was maintained separately. After the + stock was lost, +/*ey* fathers (series 24–50) were the direct or indirect product of successive backcrosses to the *ey* stock. Hence, modifiers usual to the wild type were replaced by those optimal for *ey/ey*, reducing the productive advantage of +/*ey*. This interpretation is consistent with MATHER's (1943) views, substantiated in HASKELL's (1940) study of polygene complexes affecting bristle number in scute and in wild type.

The second possibility is that after series 27, *ey/ey* ♂♂ had *ey/ey* parents which had competed successfully with +/*ey* flies for leaving offspring. In any event, the greatest increase in productivity occurs for those phenotypic classes of *ey/ey* ♂♂ which were least productive earlier, so the segregating fertility genes apparently have little effect on eye size.

TABLE 9

Effects of "line breeding" on productivity of males

Series	Line	Offspring sex	Phenotype of *ey* parents								Total	
			Small		Medium		Large		Asymmetric			
			No.*	Mean**	No.	Mean	No.	Mean	No.	Mean	No.	Mean
27, 31, 35, 39, 43, 47	*a*	♂♂	14	30.22	15	28.63	15	38.82	15	29.20	59	31.74
		♀♀	15	34.69	15	26.59	15	36.09	15	32.68	60	32.51
28, 32, 36, 40, 44, 48	*b*	♂♂	15	34.37	14	31.27	15	36.13	16	36.69	60	34.71
		♀♀	15	39.08	14	31.80	15	39.58	16	32.81	60	35.83
29, 33, 37, 41, 45, 49	*c*	♂♂	16	35.46	16	33.11	16	31.07	16	33.22	64	33.22
		♀♀	16	37.35	16	31.33	16	31.63	16	35.12	64	33.86
30, 34, 38, 42, 46, 50	*d*	♂♂	15	34.64	15	30.88	16	31.74	15	31.34	61	32.14
		♀♀	15	34.64	15	36.85	16	32.24	14	32.43	60	33.92
Total		♂♂	60	33.76	60	31.00	62	34.34	62	32.69	244	32.96
		♀♀	61	36.46	60	31.52	62	34.79	61	33.30	244	34.03

* Number of bottles.

** Mean of arcsines of the square roots of the percent. of $+/ey$ offspring.

Four breeding lines were distinguishable in the later series: *ey* progeny from series 27 were used as parents for series 31, from series 31 for series 35, etc. Table 9 shows consistently greater proportions of $+/ey$ offspring when the *ey/ey* fathers were "small" than "medium". A paired comparison test of "small" vs. "medium," pooling sons and daughters, yielded $t = 3.98$, d.f. $= 3$, $.02 < P < .05$. In contrast, the relation between "medium" and "large" differs from line to line. The statistical "line-by-parent phenotype" interaction falls a little short of the 5% significance level for both sons and daughters. Thus, while "small" is always less productive than "medium," the relative productivities of "large" and "medium" vary, "large" being more productive in early series, "medium" in the later series, on the whole. Table 10 gives an analysis of variance on data for daughters, randomly eliminating several series to ensure orthogonality. A paired comparison test of "small" vs. "medium" gave $t = 4.115$, d.f. $= 11$, $.001 < P < .01$; "medium" vs. "large" in groups 1–4, $t = 1.32$, d.f. $= 3$, $.20 < P < .30$; "medium" vs. "large" in groups 5–12, $t = 3.12$, d.f. $= 7$, $.01 < P < .02$. An analysis of sons gave similar results.

It seems warrantable to conclude that extreme smallness of eye itself reduces male productivity, probably by diminishing the frequency of copulation. STURTEVANT (1915) found that success in copulation depended partly on sight. KALMUS (1943) reported that the smaller the eye, the greater a moving object must be to be seen, since the angle between adjacent ommatidia is greater in smaller eyes. Phenotypically "medium" or "large" *ey/ey* flies could not discriminate rotating stripes as narrow as could wild type. Totally eyeless flies displayed no pattern vision at all. Extremely small-eyed males may not only lack optical equipment for locating and pursuing partners, but may behave abnormally due to brain deficiency. The sizes of the three optic glomeruli of the brain depend strictly on the size of the eye itself without obvious alteration of the rest of the brain or compensatory enlargement of the antennal ganglion (POWER 1943).

TABLE 10

Analysis of proportion of +/ey amongst daughters, mating system C. Each entry is the sum of the arcsine $\sqrt{\%+/ey}$'s from 6 bottles.

Group	Including series	Phenotypic grade of *ey* parents				Total
		"Small"	"Medium"	"Large"	"Asymmetric"	
1	10, 11, 12	231.61	194.70	213.83	241.28	881.42
2	14, 15, 19, 20	247.57	235.13	173.16	220.06	875.92
3	22, 25, 26	231.71	207.20	168.30	179.65	786.86
4	27, 28, 29	239.40	226.54	215.68	205.68	887.30
Subtotal		950.29	863.57	770.97	846.67	3431.50
5	31, 32, 33	227.56	146.74	211.57	202.35	788.22
6	34, 35, 36	214.81	193.68	241.60	182.87	832.96
7	37, 38	249.90	190.04	205.09	204.35	849.38
8	39, 40	196.37	185.07	213.43	221.98	816.85
9	41, 42	178.61	189.95	177.77	170.08	716.41
10	43, 45	235.96	177.73	189.81	210.27	813.77
11	46, 47	231.31	162.27	183.88	192.55	770.01
12	48, 49	201.08	179.20	211.18	204.19	795.65
Subtotal		1735.60	1424.68	1634.33	1588.64	6383.25
Total		2685.89	2288.25	2405.30	2435.31	9814.75

Analysis of variance

Source of variance	Groups 1–4			Groups 5–12			Groups 1–12		
	Sum sq.	d.f.	Mean sq.	Sum sq.	d.f.	Mean sq.	Sum sq.	d.f.	Mean sq.
Groups	283	3	94.3	500	7	71.5	1146	11	104.2
Grades	677	3	225.7	1049	3	349.7	1130	3	376.6
Group-by-grade	574	9	63.8	1248	21	59.4	2455	33	74.4
Total between classes	1534	15	...	2797	31	...	4731	47	...
Within classes	6263	80	78.3	11859	160	74.1	18122	240	75.5
Total betw. bottles	7797	95	...	14656	191	...	22853	287	...
Groups/within classes	$F = 1.20, P > .05$			$F < 1, P > .05$			$F = 1.38, P > .05$		
Grades/within classes	$F = 2.88, .01 < P < .05$			$F = 4.72, .001 < P < .01$			$F = 4.99, .001 < P < .01$		

BATEMAN (1948) found that the more often a male copulated, the more numerous his offspring. If a female was mated to different males in close succession, the sperm from the various partners was mixed. KAUFMANN and DEMEREC (1942) found that the sperm deposited in the last of three successive matings by an aged "virgin" male fertilized a female's five-day output of eggs. In their case, whether later sperm replaced or mixed with sperm deposited earlier depended partly on genotype. To discover whether mixing or replacement of sperm was common in mating system C, the surviving *ey/ey* mothers in series 23 were isolated in vials. Sixteen gave no offspring, 21 gave $\frac{1}{2}$ *ey/ey*: $\frac{1}{2}$ +/*ey*, and 4 gave all *ey/ey* offspring. No mixing of

TABLE 11

Male productivity coefficients $t^{♂}$ based on data from sons and daughters

Phenotype of *ey* male	Offspring sex	Series 8-30			Series 31-50		
		$t^{♂}$	95% conf. limits*		$t^{♂}$	95% conf. limits	
			Upper	Lower		Upper	Lower
Small	♂♂	−.618	−.462	−.747	−.284	−.045	−.477
	♀♀	−.576	−.397	−.723	−.401	−.185	−.576
	Pooled**	−.600			−.348		
Medium	♂♂	−.387	−.142	−.581	+.089	+.436	−.184
	♀♀	−.482	−.243	−.670	+.102	+.491	−.198
	Pooled	−.436			+.095		
Large	♂♂	−.212	+.266	−.543	−.327	−.036	−.553
	♀♀	−.113	+.325	−.431	−.276	+.030	−.475
	Pooled	−.159			−.301		
Asymmetric	♂♂	−.513	−.351	−.642	−.155	+.084	−.352
	♀♀	−.492	−.268	−.671	−.144	+.089	−.338
	Pooled	−.506			−.149		

* Based on the standard error of the mean arcsine. The mean percentage of +/*ey* offspring was transformed into an arcsine; 2σ was added or subtracted. The resulting arcsine was transformed back into a percentage, from which the corresponding upper or lower value of $t^{♂}$ was calculated.

** Obtained by weighting the estimates based on sons and on daughters by the reciprocals of the squared differences between the appropriate pairs of 95% confidence limits.

sperm was suggested. The highest proportion of *ey*/*ey* in the regular counts for series 23 were in the bottles which had the mothers producing only *ey*/*ey* after isolation. Inasmuch as the copulations resulting in the counted offspring probably occur soon after the parents are placed in the bottles, the test just cited indicates but roughly the vigor of the two kinds of males.

Table 11 gives the productivity coefficients $t^{♂}$ for each class of *ey* father:

$$t^{♂} = \frac{1 - y(4 + 3s) + 3a'(1 + s)}{2(y - a')(1 + s)} = \frac{1.012261 - y(4 + 3s)}{2y(1 + s) - .008174}$$

where s is the appropriate viability coefficient of *ey*/*ey* determined from mating system A, a' is the proportion of *ey*/*ey* phenotypically wild type, and y is the proportion of phenotypically wild type sons or daughters, averaged by bottle. The productivity coefficients estimated from sons and daughthers agree very well, and were therefore combined, weighting the separate estimates by the inverse of their variances. In series 8–30, "large" *ey*/*ey* ♂♂ are not significantly less productive than +/*ey*. "Small" males are, however, less than half as productive. "Medium" and "asymmetric" males are intermediate. In series 31–50, "small" males are 65% as productive as +; "medium," fully as productive as +; "large," 70% as productive as +; "asymmetric," 85% as productive as +. Some combinations of modifiers which enhance the fertility of *ey*/*ey* ♂♂ favor large-eyed males less than medium-eyed males.

RELATIVE PRODUCTIVITIES OF EY/EY AND +/EY FEMALES

Table 12 presents the results of mating system D, concerning the productivity of *ey/ey* ♀ ♀. There are no differences detectable between daughters in series 8–30 and in series 31–50. Because of the maternal influence on viability, some difference between sons in early and late series was anticipated. None of significance was found, comparing sons from a single class of *ey* parents, but, pooling sons from all classes of *ey* parents, $t = 1.957$, $P = .05$ of a merely chance divergence. Separate values of t^{φ} will therefore be presented for earlier and later series.

An approximate analysis of variance disclosed no component of variance of male arcsines assignable to class of *ey* parents ($F = 0.69$, d.f. = 3 and 371, $P > .05$). However, a significant component of the variance of female arcsines was due to maternal grade ($F = 3.02$, d.f. = 3 and 372, $.01 < P < .05$). For the comparison of daughters of "small" vs. "medium" parents, $t = 2.20$; "small" vs. "large," $t = 2.36$; "large" vs. "asymmetric," $t = 2.06$—all significant at the 5% level. The close parallelism between sons and daughters strongly suggests real differences between classes of *ey* mothers, whose borderline significance has been somewhat exaggerated in daughters and depressed in sons by accidents of sampling. A paired comparison test of "medium" vs. "small" in both sexes and series groups (d.f. = 3) yielded $t = 3.30$, $.02 < P < .05$; between "medium" and "asymmetric", $t = 3.42$, $.02 < P < .05$. Productivity is highest in "medium" and "large" mothers, lowest in "asymmetric."

When all offspring eclosing during a specified period were counted (series 7–40), there was no significant relationship between productivity of *ey/ey* ♀ ♀ relative to +/*ey* ♀ ♀ and total number of sons per bottle, except that "small" females had relatively more sons when the total per bottle was low. The incidence of large and small counts was distributed at random over the classes of *ey* parents and through the early and late series (8–30 vs. 31–50).

No effect of "line breeding" after series 30 was found in sons (table 13). However, "large" parents consistently produced higher proportions of *ey/ey* daughters than

TABLE 12

Comparison of arcsin $\sqrt{\%+/\text{ey}}$ *in series 8–30 and series 31–50 mating system D, for sons and daughters*

Phenotype of *ey* parents	Offspring sex	Series 8-30			Series 31-50			S.e. of diff. of means	STUDENT'S *t*	*P*
		No. bottles	Mean % +/*ey*	Mean arcsine	No. bottles	Mean % +/*ey*	Mean arcsine			
Small	♂♂	43	33.03	34.72	55	30.69	32.95	1.631	1.087	> .05
	♀ ♀	42	32.33	34.31	55	32.74	34.69	1.290	0.297	> .05
Medium	♂♂	47	30.94	33.04	55	29.28	32.60	1.419	0.312	> .05
	♀ ♀	47	30.27	32.78	55	28.78	32.05	1.486	0.495	> .05
Large	♂♂	32	31.92	33.96	54	29.23	32.47	1.573	0.949	> .05
	♀ ♀	32	30.75	33.18	54	27.92	31.46	1.737	0.986	> .05
Asymmetric	♂♂	37	34.62	35.86	53	30.86	33.08	1.733	1.605	> .05
	♀ ♀	37	34.66	35.66	54	31.47	33.68	1.699	1.167	> .05
Pooled	♂♂	159	32.56	34.33	217	30.01	32.77	0.795	1.957	≃ .05
	♀ ♀	158	31.95	33.93	218	30.23	32.97	0.779	1.233	> .05

TABLE 13

Effects of "line breeding" on productivity of females

Series	Line	Offspring sex	Phenotype of *ey* parents								Total	
			Small		Medium		Large		Asymmetric		No.	Mean
			No.*	Mean**	No.	Mean	No.	Mean	No.	Mean		
27, 31, 35, 39, 43, 47	*a*	♂♂	15	32.90	15	31.89	15	32.89	15	34.93	60	33.15
		♀♀	15	35.19	15	31.65	15	29.26	15	33.65	60	32.44
28, 32, 36, 40, 44, 48	*b*	♂♂	16	35.61	16	33.64	15	33.18	15	38.19	62	33.14
		♀♀	16	35.45	16	34.98	15	32.34	16	39.05	63	35.50
29, 33, 37, 41, 45, 49	*c*	♂♂	16	31.24	16	32.76	16	31.46	15	33.20	63	32.15
		♀♀	16	34.00	16	31.98	16	32.84	15	33.27	63	33.02
30, 34, 38, 42, 46, 50	*d*	♂♂	15	32.83	16	33.36	16	33.10	15	29.00	62	32.11
		♀♀	15	34.19	16	31.00	16	31.85	15	29.14	62	31.54
Total		♂♂	62	33.15	63	32.93	62	32.65	60	33.83	247	33.13
		♀♀	62	34.71	63	32.42	62	31.60	61	33.86	248	33.14

* Number of bottles.

** Mean of arcsines of the square roots of the percentage of $+/ey$ offspring.

did "small" parents. The daughter difference between lines borders on significance at the 5% level, suggesting genetic differences established during line breeding. The lack of "line-by-phenotype" interaction indicates less divergence in female than in male fertility modifiers. Marked differences between lines existed only for "asymmetric" where $F = 5.99$, d.f. $= 3$ and 57, $P < .01$.

The formula for the productivity coefficient l^{φ} of females must be modified as follows for maternal influence on male viability:

Mother genotypes:	$+/ey$	ey/ey
Offspring zygotes	$1\ +/ey{:}1\ ey/ey$	$(2 + 2l)\ ey/ey$
Offspring adults:	$1\ +/ey{:}(1 + s_B)ey/ey$	$(2 + 2l)(1 + s_A)ey/ey$

$$\text{or, } \frac{1}{4 + (2s_A + s_B) + 2l(1 + s_A)} +/ey{:}\ \frac{3 + (2s_A + s_B) + 2l(1 + s_A)}{4 + (2s_A + s_B) + 2l(1 + s_A)}\ ey/ey$$

where s_A refers to ey/ey offspring of ey/ey mothers and s_B, ey/ey offspring of $+/ey$ mothers. The proportion of adults appearing wild type is

$$y = \frac{1 + a'[3 + (2s_A + s_B) + 2l(1 + s_A)]}{4 + (2s_A + s_B) + 2l(1 + s_A)},$$

where a' is the proportion of ey/ey flies overlapping wild type. Then

$$l^{\varphi} = \frac{1 + a'[3 + (2s_A + s_B)] - y(4 + s_B + 2s_A)}{2(1 + s_A)(y - a')}$$

$$= \frac{1.012261 - y(4 + s_B + 2s_A)}{2y(1 + s_A) - .008174}.$$

Table 14 presents l^{φ} for each class of *ey* mother. All were more productive in the later series; STUDENT's t for the paired comparisons between early and late is 3.28,

TABLE 14

Female productivity coefficients t^{φ} based on data from sons and daughters

Phenotype of *ey* female	Offspring sex	Series 8-30			Series 31-50		
		t^{φ}	95% conf. limits*		t^{φ}	95% conf. limits	
			Upper	Lower		Upper	Lower
Small	♂♂	−.323	−.105	−.500	−.201	+.047	−.403
	♀♀	−.211	+.011	−.395	−.234	−.080	−.369
	Pooled**	−.269			−.224		
Medium	♂♂	−.210	+.062	−.425	−.111	+.026	−.233
	♀♀	−.079	+.191	−.299	+.021	+.256	−.175
	Pooled	−.145			−.076		
Large	♂♂	−.265	+.006	−.478	−.108	+.080	−.269
	♀♀	−.111	+.247	−.386	+.087	+.340	−.124
	Pooled	−.208			−.037		
Asymmetric	♂♂	−.401	−.179	−.577	−.212	+.041	−.417
	♀♀	−.341	−.080	−.548	−.150	+.070	−.348
	Pooled	−.376			−.183		

* Based on the standard error of the mean arcsine. The mean percent of $+/ey^1$ offspring was transformed into an arcsine; 2σ was added or subtracted. The resulting arcsine was transformed back into a percentage, from which the corresponding upper or lower value of t^{φ} was computed.

** Obtained by weighting the estimates based on sons and on daughters by the reciprocals of the squared differences between the appropriate pairs of 95% confidence limits.

d.f. = 3, $.02 < P < .05$. The son and daughter estimates of t^{φ} agree better for "small" and "asymmetric" than for "large" and "medium." The productivity of the last two appears greater when daughters rather than sons are considered; STUDENT's t of the paired comparisons between sons and daughters is 3.97, d.f. = 7, $.001 < P < .01$. Perhaps the rate at which "medium" and "large" females lay eggs increases relative to $+/ey$ during the first days of oviposition. Throughout the experiment, daughters eclosed earlier than sons. The eggs developing into sons may be laid earlier than those developing into daughters included in the same count. (Any difference in development rate between ey/ey and $+/ey$ would be subsumed in s and would not affect t.)

In series 8–30, ey/ey ♀♀ ranged from 62% to 86% as productive as $+/ey$ ♀♀ (using the pooled estimates); in series 31–50, from 78% to 96% as productive.

The circus movements of "asymmetric" females may hinder oviposition. Otherwise, there is no *a priori* reason for presuming that female eye size itself influences either copulation or oviposition. Possibly, the smaller optic ganglia in the small-eyed fly might impair egg-laying behavior—as in choosing an oviposition site. Any other correlation between productivity and eye size must result from a less direct effect of the *ey* allele, or from the segregation of modifiers acting early in the sequence of events initiated by *ey*. When the *ey* locus acts to influence eye size is not known, but the optic disk is smaller in ey/ey than in normal from the beginning (CHEN 1929). The temperature-effective period of *eyeless* (ey^2) extends from before hatching until the optic disk is differentiated (BARON 1935). Eye size can be affected as early as

four hours after the egg is laid, two hours after blastoderm formation (HOWLAND and CHILD 1935), though not before the maturation divisions of the maternal nucleus (HOWLAND and SONNENBLICK 1936). Nevertheless, how much optic disk material will form facets is not completely determined until puparium formation (STEINBERG 1944).

DISCUSSION

Unless modifiers independently alter eye size and viability or productivity in the *ey*/*ey* fly, the consequences of differential viability or productivity of different phenotypic classes of a single genotype can be predicted. It has been assumed here that the + allele is fully dominant, and, further, that the selection coefficients are independent of the frequency of the major gene (in this case, of *ey*). When there are different viability coefficients for the various phenotypic classes of the recessive genotype, the net viability coefficient for one sex is the weighted average of the coefficients of the different classes; for the genotype as a whole, $\bar{s}$, the unweighted average of the coefficients for the two sexes. The same methods of averaging apply to the productivity coefficient, t. In a random-breeding population, the change in the zygotic frequency of the recessive allele in one generation is given by the approximate expression

$$\delta q = q^2(1 - q)(\bar{s} + \bar{t}),$$

if $\bar{s}$, $\bar{t}$ and q are small enough that their products are negligible.

Thus, any situation which increases either $\bar{s}$ or $\bar{t}$—i.e., decreases their negative value—will favor a slower decline, or an increase, in the proportion of recessive individuals. Modifiers which increase those categories of recessive individuals with higher values of either s or t will be selected for so long as there is an appreciable proportion of recessive individuals. The speed with which such modifiers accumulate can be judged from the various indices of segregation which in the present experiment rose from zero at the beginning to a significant extent in series 8–20 and jumped markedly in the 57-day interval between series 20 and 21.

Eye size in the *ey*/*ey* fly seems to have no effect on the viability coefficient since the proportion of *ey*/*ey* progeny did not depend on the *ey* parents' phenotype in mating systems A and B, although the average eye grade of progeny did depend on it. Modifiers of *ey* can therefore be expected to derive their greatest selective advantage from improving male productivity by increasing eye size. Modifiers directly affecting productivity independently of eye size, but as a part of the optimal complex of polygenes in the *ey*/*ey* genotype, undoubtedly contributed to the general increase in male productivity in the latter half of the experiment.

SUMMARY

Whether a deleterious recessive gene of variable expression is subjected to selection the intensity of which is related to the phenotypic departure from wild type was tested for the ey^1 allele. Selection was analyzed into two components: viability and productivity. Two stocks initially differing only in fourth chromosomes were the source of the ey^+ and ey^1 alleles.

1. The isogenic strain of *ey*^{1} gradually accumulated an abundant supply of modifiers for eye size, as shown by greatly increased variance, the midparent-offspring correlation rising from an initial value of zero to .20 in the first quarter and to .46 in the last quarter of the experiment, and the correlation between right and left rising from zero to .25 and then to .46 in these periods.

2. Eyeless males were at a selective disadvantage of 13% in viability; females, 18%, in both cases independently of eye size of *ey* parents—either mother or father. The viability of eyeless flies can not be affected by their own eye sizes in view of the parent-offspring correlation. There is a slight maternal effect on the viability of eyeless males.

3. Eyeless males were at a severe disadvantage when competing with $+/ey$ ♂♂ to leave offspring. Small-eyed males were at a greater disadvantage (60% in the first half, 35% in the second half of the experiment) than medium-eyed males (44% in the first half, 0% in the second), while asymmetric males were intermediate, probably a direct result of poor vision. Larger-eyed males are in some lines at a greater, and in other lines at a less, disadvantage than medium-eyed males. In general, the larger-eyed males were superior to other *ey* males in the first half but inferior in the second half of the experiment, probably because modifiers were on the whole selected for adjustment to the "medium" type in the breeding system followed in the latter.

4. Eyeless females were at some productive disadvantage to $+/ey$ ♀♀ (ca. 20%). Small-eyed and asymmetric females were at a greater disadvantage than medium-eyed and large-eyed females. There was also improvement during the course of the experiment. All of these effects are less striking than in the case of male productivity. They may reflect more indirect pleiotropic effects of *ey*^{1} and its modifiers.

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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